

**Clinical Significance of Adenosine Signaling Deficits in Schizophrenia Pathology: From
Theoretical Foundations to Symptomatology**

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Clinical Significance of Adenosine Signaling Deficits in Schizophrenia

Pathology: From Theoretical Foundations to Symptomatology

Xiang Zhou

University of Pittsburgh, 2018

Schizophrenia (SZ) is a devastating mental illness characterized by complicated pathophysiology. Adenosine theory was proposed recently and showed the potential to combine classic theories into one common pathway. It is hypothesized that the reduction of adenosinergic activity in CNS could serve as a possible common explanation for the alterations of dopaminergic and glutamatergic neurotransmission in SZ. Based on previous reports and preliminary data, adenosine signaling might be an interesting and meaningful target to study. This dissertation thus was dedicated to test adenosine theory and to explore its clinical significance in SZ.

This dissertation consists of four parts. First, we tested if levels of analytes in adenosine signaling was altered in SZ patients. We observed significant level changes of adenosine, adenosine deaminase (ADA) and inosine in SZ patients. We also observed disrupted connections between adenosine and ADA in SZ patients when comparing with that in controls. Second, we tested if such deficits could be affected by antipsychotic treatments. In this part results implied that antipsychotics could have an impact on adenosine signaling. However, treatments cannot fully restore the altered levels of those analytes. Third, we tested associations between the adenosine signaling within purine pathway and with other important biochemical pathways in SZ pathology.

A homeostasis imbalance of purine pathway were observed and the pathway was shifted towards the direction in favor of uric acid production. We also observed that reduced uric acid levels were linked to elevated levels of oxidative stress. For inter-pathway correlations, we found significant correlations between adenosine levels and red blood cell membrane defects. We also observed inverse correlation between adenosine and homovanillic acid. Fourth, significant correlations were observed between analytes' levels and positive symptoms as well as between analytes' levels and global functioning scale. Findings here suggested that levels of adenosine and ADA in plasma and CSF may have important clinical implications.

In summary, adenosine signaling deficits were suggested to be present in SZ patients and can be linked to classical SZ theories. Also, findings revealed the clinical significance of adenosine signaling and may shed light on the endeavors for new antipsychotics R&D.

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PREFACE

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ABBREVIATIONS

SZ:	schizophrenia
PET:	positron emission tomography
NMDAR:	N-methyl-D-aspartate receptor
PNS:	peripheral nervous system
CNS:	central nervous system
PLA ₂ :	phospholipases A ₂
DRs:	dopamine receptors;
LTP:	long-term potentiation
LTD:	long-term depression
AR:	adenosine receptor
ADK:	adenosine kinase
ADA:	adenosine deaminase
DNA:	deoxyribonucleic acid
ROS:	reactive oxygen species
FEANS:	first-episode antipsychotic naïve schizophrenia patients
AODS:	antioxidant defense system
FA:	fatty acid
FFA:	free fatty acid
RBC:	red blood cell

HVA:	homovanillic acid
PAH:	phenylalanine hydroxylase
CSF:	cerebrospinal fluid
FEANS:	first episode antipsychotic naïve schizophrenia patient
HC:	healthy control
DC:	disease control
BL:	baseline
ELISA:	enzyme-linked immunosorbent assay
ANOVA:	analysis of variance
NO:	nitric oxide
MDA:	malondialdehyde
TAS:	total antioxidant status
HPLC-CMEAS	high-pressure liquid chromatography coupled with electrochemical coulometric array system
GC:	gas chromatography
Phe:	Phenylalanine
Tyr:	Tyrosine
DSM:	diagnostic and statistical manual of mental disorders
BIC:	schwarz's bayesian criterion
CASH:	comprehensive assessment of symptoms and history
BPRS:	brief psychiatric rating scale

SAPS: scale for the assessment of positive symptoms

SANS: scale for the assessment of negative symptoms

GAS: global assessment of functioning score

1.0 INTRODUCTION

1.1 SCHIZOPHRENIA AND CLASSICAL THEORIES IN EXPLAINING ITS PATHOLOGY

Schizophrenia (SZ) is a debilitating mental disorder with a global prevalence from 0.4% to 0.7% (Bhugra 2005, van Os and Kapur 2009). The active phase of SZ typically begins in late adolescence or early adulthood and lasts a long time to senior ages (National Institute of Mental Health 2016, American Psychiatric Association 2013). Common symptoms fall into three major categories: positive, negative and cognitive. Positive symptoms are those that are present in people with schizophrenia and that normal people do not experience. They include delusions, hallucinations, disordered thoughts, movements and behaviors. Negative symptoms are deficits or reduction in normal emotional responses, experiencing of pleasure, motivation and verbal productivity. They include “flat affect” (reduced expression of emotions via facial expression, gesture or voice tone); anhedonia (reduced feelings of pleasure in everyday life); motivation deficits such as difficulty beginning and sustaining activities and alogia (reduced speaking and reduced content of speech). Cognitive symptoms refer to deficits in cognitive abilities including executive functioning, immediate and sustained attention and working memory. It is undeniable that SZ imposes a massive personal and economic burden to the individuals with

schizophrenia, their families and society. Social problems caused by SZ, such as homelessness, long-term unemployment and poverty are common (American Psychiatric Association. 2013, Foster et al. 2012).

The study of SZ's etiology and symptomatology has been pursued for nearly a century. However, SZ is a disease of complicated pathology. In addition to its heterogeneous clinical presentation and course of illness, the nature and origin of the pathology is unclear and unlikely to be uniform. With regard to observed biochemical abnormalities, SZ has been attributed to multiple metabolic defects originating from different biochemical pathways. Previous studies have linked SZ to widespread structural and functional brain alterations, including multiple neurotransmitter pathway disruptions (Karam et al. 2010, Walsh et al. 2008, Brisch et al. 2014, Kerne, 2009), white matter changes (Kubicki et al. 2005, Lener et al. 2014) and prefrontal- limbic network dysfunctions (Weinberger et al. 1992, Weinberger et al. 1988). Based on previous findings, there are four classical biochemical theories that aim to explain SZ's pathology: Dopamine, glutamate, phospholipid and serotonin hypothesis (Table 1). However, none of them can fully explain SZ's pathophysiology. While there are disparate biological findings and theories reported in SZ, accumulating evidence suggests that there might be up-stream factors (Howes and Kapur, 2009). Alterations of these up-stream factors by SZ eventually lead to down-stream biochemical defects which have been reported that point to striatal dopamine hyperfunction (Meltzer and Stahl. 1976, Seeman. 1987), NMDAR hypofunction (Kim et al. 1980, Coyle. 1996, Moghaddam and Javitt, 2012), cerebral serotonin overdrive (Eggers. 2013) and increased lipolysis of cell membranes (Horrobin. 1998).

Table 1. Classical theories in explaining schizophrenia's pathology

Theory	References	Claim	Limitation
Dopamine	Meltzer and Stahl, 1976; Seeman, 1987; Howes and Kapur, 2009.	A striatal hyperactivity of the dopaminergic signaling leads to SZ pathology and symptomatology.	1. PET data have technical limitations. 2. Improvement in psychosis without modulating dopamine system by antipsychotics such as LY2140023 (NMDAR agonist).
Glutamate	Kim et al. 1980; Coyle, 1996; Moghaddam and Javitt, 2012.	Reduced NMDAR activity on inhibitory neurons leads to the disinhibition of synaptic glutamate activity in the prefrontal cortex and causes SZ symptoms.	1. Weak in correlating & explaining positive symptoms. 2. Several subgroups of SZ patients are no responsive to NMDAR agonists.
Lipid	Horrobin, 1998	Due to increased activity of PLA ₂ , an increased break down of cell membranes in PNS and CNS causes SZ symptoms.	1. Limited efficacy of lipid-modulating drugs on SZ symptoms. 2. Weak in correlating & explaining negative symptoms.
Serotonin	Eggers, 2013	Chronic and widespread stress-induced serotonergic overdrive in the cerebral cortex is the basic cause of the disease.	1. Weak in correlating & explaining negative and cognitive symptoms. 2. Second-generation antipsychotics on serotonin pathway are also affecting dopaminergic signaling.

Abbreviations: NMDAR = N-methyl-D-aspartate receptor; PET = positron emission tomography; SZ = schizophrenia; PLA₂ = phospholipases A₂; PNS = peripheral nervous system; CNS = central nervous system.

1.2 NEW EMERGING HYPOTHESIS AND ADENOSINE SIGNALING

Adenosine hypothesis of SZ was first proposed in 2006 and further developed recently (Lara et al. 2006, Boison et al. 2012). Specifically, the new hypothesis suggests that a specific dysfunction in the purinergic system would result in reduced adenosinergic activity (Figure 1)

in CNS, which could serve as a possible common explanation for the imbalance of dopaminergic and glutamatergic neurotransmission that is characteristic of SZ.

There are two major sources of evidence to support the new emerging hypothesis. The first source of evidence comes from solid animal pharmacology data. Previous studies have revealed adenosine's effect on monoamine pathways in the CNS (Matos et al. 2015, Pardo et al. 2012, Salmi et al. 2005). Specifically, activation of A_{2A}R inhibits both NMDAR and D₂Rs while activation of A₁Rs inhibits D₁Rs and enhances NMDARs. Those manipulations correlate with motor activity, learning and memory in animal models (Matos et al. 2015, Pardo et al. 2012). The molecular basis of the adenosine-based manipulation on dopaminergic and glutamatergic neurotransmission has been summarized in Figure 2. On the other hand, however, there is no evidence that dopamine or other neurotransmitters can regulate adenosine levels in the CNS.

The second source of evidence implies that the manipulation of adenosine signaling could have potential clinical significance in SZ pathology, as discussed below. First, augmentation of adenosine by pharmacologic inhibition of adenosine kinase (ADK), the key enzyme of adenosine clearance, exerts antipsychotic-like activity in mice (Shen et al. 2012). In contrast, overexpression of ADK in transgenic mice was associated with attentional and learning impairments which linked to SZ (Shen et al. 2012, Yee et al. 2007). Second, it is reported that atypical anti-psychotics can up-regulate adenosine receptors (A_{2A}R) and improve sensory gating in SZ patients (Zhang et al. 2012). Third, one clinical study suggested that purinergic signaling modulator adjuvant therapy (allopurinol, xanthine oxidase inhibitor) is

more beneficial in SZ psychopathology in comparison with placebo, especially in the treatment of refractory positive symptoms (Brunstein et al. 2005).

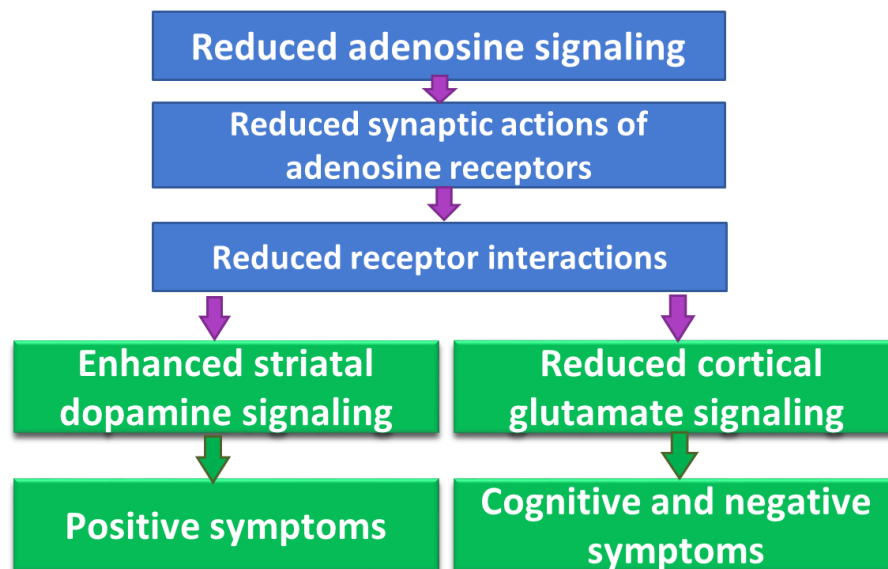


Figure 1. Reduced adenosine signaling and proposed underlying mechanisms of SZ pathophysiology.

Contents in green boxes refer to established relationships and contents in blue boxes refer to the postulated hypothesis.

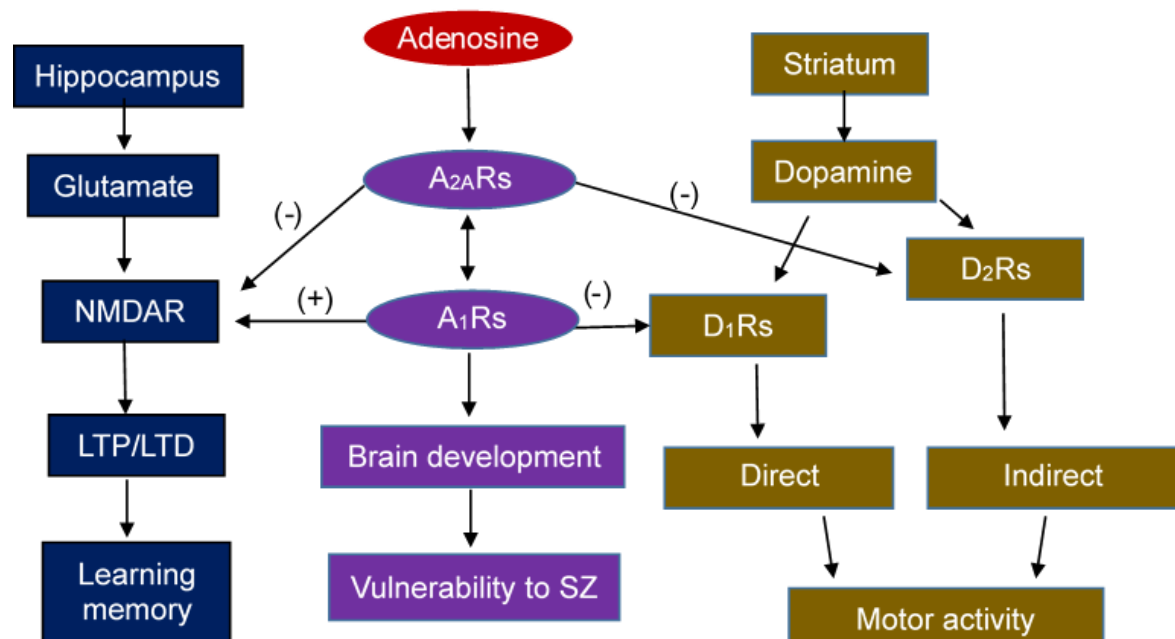


Figure 2. The molecular basis and pathways of adenosine-based manipulation of glutamatergic and dopaminergic neurotransmission in schizophrenia pathology.

Abbreviations: D₁Rs = dopamine 1 receptors; LTP = long-term potentiation; LTD = long-term depression; NMDAR = N-methyl-D-aspartate receptor; SZ = schizophrenia.

Based on findings mentioned above, it is suggested that adenosine signaling might be an interesting target to study. Thus, a brief introduction about adenosine signaling is provided here. Adenosine is a purine nucleoside composed of a molecule of adenine that is attached to a ribose sugar molecule (ribofuranose) moiety via a β -N₉-glycosidic bond (Morton and Judith. 2012, Buckingham. 1987, Taylor and Francis. 2000). Adenosine may be formed intracellularly in both neuronal and glial cells (Latini and Pedata. 2001, Meghji et al. 1989) from degradation of adenosine monophosphate, and then exit via bi-directional nucleoside transporters, or extracellularly by the metabolism of released nucleotides (Zimmermann. 1996). In the brain, adenosine is an inhibitory neurotransmitter. This means, adenosine could act as a central nervous system (CNS) depressant. The increase of adenosine activity can lead to the decreased release and activity of other neurotransmitters such as dopamine and norepinephrine (Ferré et al. 1997, Burgdorf et al. 2001, Rongen et al. 1996), while no evidence showed that dopamine or norepinephrine can have an impact on adenosine levels or adenosine signaling activity in the brain. In normal conditions, adenosine promotes sleep and suppresses arousal (Basheer et al. 2004, Scammell et al. 2001, Gallopin et al. 2005).

After being released from intracellular stores into circulation, adenosine is broken down to inosine by adenosine deaminase (ADA) which presents in both CNS and peripheral tissues (Lovatt et al. 2012). There are two recognized isoforms of ADA in the CNS: ADA1 and ADA2. The metabolism and physiological transportation of these two isoforms in the brain and CSF are still not clear. Since the ratio and distribution of these two isoforms in the brain are poorly studied, the K_m of ADA for adenosine is in the range (depends on ratios of isoforms) of 25–

150 μM with a V_{max} at $3.6 \times 10^{-9} \mu\text{M/s}$ under normal adenosine concentration levels (Ford et al. 2000, Singh and Sharma. 2000). This K_m is much higher than adenosine concentrations under normal physiologically condition which is below 1 μM (Conlay et al. 1997). Therefore, the reaction from adenosine to inosine via ADA might be substrate-limited. Currently, the full physiological role and regulation of ADA is not yet completely understood (Cristalli et al. 2001). There are some evidence suggested that ADA can be regulated by cytokines through fine immunoregulatory mechanism of NF- κ B signaling in leukocytes. Specifically, it is reported that interleukin-2 and interleukin-12 can up-regulate ecto-ADA and CD26 (a lymphocyte marker that can anchor ADA on the T cell surface) expression while interleukin-4 led to down-regulation of lymphocyte surface ADA without modifying the level of CD26 (Cordero et al. 2001, Wiginton et al. 1991). However, no evidence has linked adenosine, dopamine or any other neurotransmitters with the regulation of ADA expression or activity. Current assays on ADA (including the assay in this dissertation) was measuring on its activity which expressed by International Unit (I/U or U). One Unit is the amount of enzyme that catalyzes the reaction of 1 μmol of substrate per minute. The normal reference range of ADA activity is from 15.0-23.2 I/U in the plasma (Al-Shammary et al. 1993) and 0-9 I/U in the CSF (Corral et al. 2004). In peripheral tissues, both adenosine and its metabolite (inosine) can cross through the blood-brain barrier via purine transporters located on brain capillary endothelial cells (Cornford and Oldendorf. 1975, Pardridge. 2005). However, few studies have explored the relationship of metabolite and enzyme levels in the CNS versus peripheral tissues.

Adenosine signaling and adenosine receptors (ARs) have been well studied in past decades. All ARs subtypes (A_1 , A_{2A} , A_{2B} , and A_3) are G-protein-coupled receptors. There are two major types of ARs (A_1R and A_{2AR}) enriched in the CNS. The A_1 receptors couple to $G_{i/o}$ and decrease adenylate cyclase levels, while the A_2 receptors couple to G_s , which enhances adenylate cyclase activity. Many important biochemical processes are controlled by ARs and their subsequent regulation of adenylate cyclase, such as cell proliferations (Pardee. 1989, Stork and Schmitt. 2002, Igata et al. 2005, Granata et al. 2007), immune responses (Deaglio et al. 2007, Haskó & Cronstein. 2004, Sitkovsky and Lukashev. 2005, Haskó et al. 2008) and lipid metabolism (Bricker and Levey. 1972, Park et al. 2008, Koupenova et al. 2012, Guo et al. 2012). However, the potential for adenosine signaling alterations in SZ patients, either in plasma or CNS, is still unclear today. Furthermore, as mentioned above, while atypical anti-psychotics can up-regulate adenosine receptors (A_{2AR}) and alleviate clinical symptoms, the clinical treatment effects on adenosine, inosine and ADA activity levels in SZ patients are still unknown and urgently need to be studied. Considering the new adenosine hypothesis that is introduced above, we believe that studying adenosine signaling in SZ pathology would be helpful in unveiling SZ's pathophysiology and offering useful directions in developing new treatment strategies.

1.3 PURINE PATHWAY IN SCHIZOPHRENIA

Biochemically, adenosine and its metabolites belong to a group of molecules referred to as purines. Adenosine signaling is a step in the process of purine metabolism (Figure 3). Purines are critical in building up blocks of genetic codes and therefore the foundation of life, as represented by deoxyribonucleic acid (DNA). In other words, purines are central to the maintenance and reproducible existence of our nucleotide-protein system, which produces cells and tissues (Fredholm et al. 2010). According to the adenosine hypothesis, in SZ, adenosine signaling deficits are caused by a purine system dysfunction (Lara et al. 2006). Therefore, it would be interesting to study the relationship between a possible disturbance of the purine pathway and adenosine signaling in SZ.

The disturbance of the purine pathway in SZ was firstly reported in the 1950s (Kishimoto, 1956). By using paper chromatography, Kishimoto found a significant difference in the absorbance of purines among individuals with SZ and normal control subjects both in serum and in brain specimen. Interestingly, our lab also found significant alterations of several purine metabolites in plasma among first-episode antipsychotic-naïve schizophrenia patient (FEANS) such as uric acid and xanthosine (Yao et al. 1998, Yao et al. 2010). However, adenosine signaling was not measured at that time; thus, the question of whether alterations of the purine pathway can cause the compensatory shift from adenosine to uric acid (and eventually lead to the adenosine signaling deficit) was not addressed in those studies. Therefore, it is proposed here that the addition of adenosine signaling as well as other metabolites (guanosine, guanine,

xanthine and hypoxanthine) into the model (Figure 3) would fill in an important part of the puzzle.

As mentioned above, our previous study showed a significant decrease of uric acid in the plasma of FEANS patients (Yao et al. 1998). Since uric acid is an important antioxidant and accounts for up to 65% of reactive oxygen species (ROS) clearance in the body (Wayner et al. 1987, Miller et al. 1993), it is hypothesized that the uric acid deficit is due to the increase of ROS levels and the breakdown of antioxidant defense system (AODS). The increase of ROS levels and the breakdown of AODS reflects an elevated level of oxidative stress in SZ. In fact, based on previous publications, oxidative stress plays a significant role in SZ pathology. Several published studies indicate that there is an increased level of major oxidative stress markers (Boscovic et al. 2011, Emiliani et al. 2014, Akyol et al. 2002) and a breakdown of the antioxidant system (Dadheech et al. 2006, Yao et al. 2011, Mukherjee et al. 1996, Ranjekar et al. 2003) in SZ patients. Clinical studies also successfully correlate oxidative stress to the positive (Pavlović et al. 2002, Fendri et al. 2006), negative (Gunes et al. 2017, Wood et al. 2009) and cognitive symptoms (Maas et al. 2017, Hardingham and Do. 2016) in SZ. These findings motivated us to explore the correlation of oxidative stress with purine pathway alterations (especially the reduction of uric acid) in SZ. In summary, we hypothesize that oxidative stress causes uric acid reduction and leads to a homeostatic imbalance in the purine pathway which eventually results in adenosine signaling deficits.

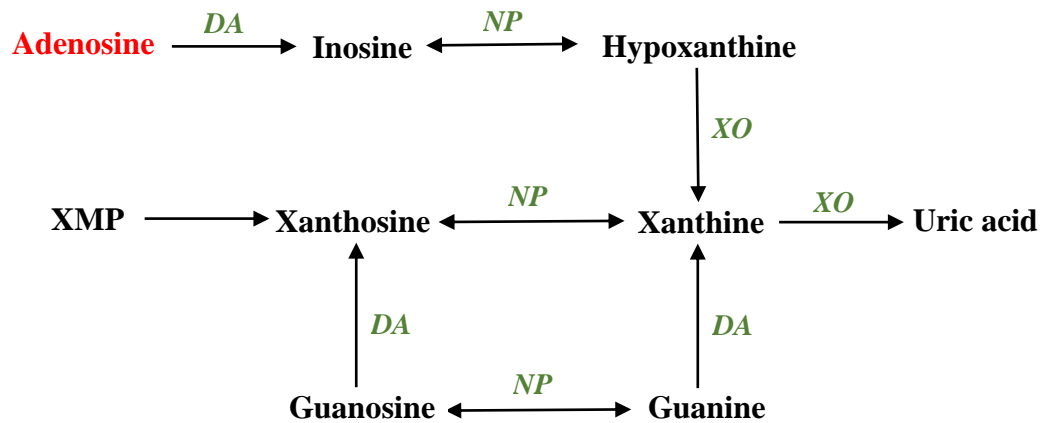


Figure 3. Purine pathway metabolism.

Abbreviations: NP = nucleoside phosphorylase; DA = deaminase; XO = xanthine oxidase; XMP = xanthosine monophosphate.

1.4 LIPID THEORY AND ADENOSINE SIGNALING

As a major component of myelin, phospholipids play a crucial role in maintaining myelinated neuronal axons. Several studies have shown that at least 70% dry mass of both CNS and PNS myelin is formed by lipids (Baumann and Pham-Dinh, 2001, Chrast et al. 2010). The classic lipid theory of SZ claims that an increased breakdown of neuron cell membranes in PNS & CNS results in SZ symptoms. Specifically, previous findings (Gattaz et al. 1990, Pettegrew et al. 1991, Horrobin et al. 1991, Noponen et al. 1993) suggested that in both CNS and peripheral tissues, SZ patients have an increased breakdown of membrane phospholipids due to increased PLA₂ activity. These observations have led to the hypothesis that the increased breakdown of phospholipids may cause dyslipidemia, impair myelinated neurons and contribute to SZ pathology. Currently there are few studies that have compared the levels of membrane

phospholipids, the resulting free fatty acids (FFAs) after breakdown, cholesterol and triglycerides all together in SZ patients versus normal controls. Therefore, an investigation of lipid metabolism in SZ is proposed here to investigate the biochemical causes of dyslipidemia in SZ pathology.

On the other hand, adenosine's role in mediating lipid metabolism--especially in lipolysis and insulin activity--has been well-studied in animal models. ARs are present within pancreatic islets and are suggested to mediate insulin secretion. Specifically, low adenosine concentration in rat islets can decrease glucose-induced insulin secretion while elevated concentration can increase secretion. This regulation is via A₁ receptor (Szkudelski and Szkudelska. 2015, Campbell et al. 1982). Over-expression of adipocyte A₁ receptor in transgenic mice prevented the occurrence of insulin resistance induced by a high fat diet (Dong et al. 2001). In rat fat cells, adenosine increased insulin activity, glucose transport maximal response and fatty acid synthesis (Joost and Steinfelder. 1982, Koupenova et al. 2013) via the A₁ receptor. Also, adenosine enhanced effect of insulin on the inhibition of lipolysis via the A₁ receptor (Schwabe et al. 1974, Johansson et al. 2008). The mechanism by which A₁ receptor enhances insulin sensitivity is mediated by the activation of cAMP-dependent protein kinase (Koupenova et al. 2013). These findings suggest that adenosine may play a critical role in lipid metabolism.

Based on these findings, the hypothesis was made that decreased adenosine signaling in SZ might cause increased lipolysis of membrane phospholipids and the observed dyslipidemia. A recent study found that dyslipidemia is linked to a down-regulated adenosine

signaling (Liu et al. 2014). In Liu's study, abnormal lipid metabolism induces down-regulation of ATP synthase via the Akt phosphorylation pathway in a high-fat diet mouse model. In human studies, despite numerous publications that implicate a disturbance of lipid metabolism (such as increased membrane lipolysis) in SZ patients, few studies have demonstrated correlation between lipid metabolism and adenosine signaling. Therefore, partial support for the lipid theory of SZ would be provided by investigation of the correlation of membrane lipolysis with adenosine signaling among individuals with SZ.

1.5 DOPAMINE THEORY AND ADENOSINE SIGNALING

After more than fifty years' revision, the current dopamine theory is now depicting a complex picture for schizophrenia. It links pregnancy complications, stress, drug use and genes to an increased striatal dopaminergic function (Howes & Kapur. 2009). With regard to dopamine metabolism, we are interested in dopamine's two major precursor amino acids (phenylalanine and tyrosine), and also its major metabolite--homovanillic acid (HVA).

Phenylalanine and tyrosine are precursor amino acids in the biosynthesis of dopamine. In the brain, the enzyme phenylalanine hydroxylase (PAH) converts phenylalanine to tyrosine. Previous findings (Table 2) implicate a dysregulation of dopamine synthesis in SZ. Genetic studies have also suggested that PAH gene polymorphisms confer susceptibility to schizophrenia (Talkowski et al. 2009) and modify features of psychotic disorders (Bergen et al. 2010). On the other hand, HVA is the final product of dopamine metabolism. Plasma HVA

has long been believed to be a peripheral index of subcortical dopaminergic activity (Pickar et al. 1988, Beuger et al. 1996). Since the dopamine theory suggests an increased striatal dopaminergic synthesis and transmission in SZ patients (Lindström et al. 1999, Kegeles et al. 2010), it is expected that plasma HVA levels will be increased in SZ patients. In fact, a higher level of plasma HVA has been found in prodromal states of SZ (Sumiyoshi et al., 2000). Furthermore, significant correlations between plasma HVA level and SZ positive and negative symptoms have been reported (Pickar et al. 1986, Davis et al. 1985, Joel et al. 1993). These findings have made HVA as well as phenylalanine and tyrosine interesting targets to study.

As mentioned in section 1.2, adenosine signaling has an inhibitory effect on DRs that are predominantly distributed in the striatal area (Missale et al. 1998). To test the adenosine hypothesis, we would like to investigate the hypothesized correlation of adenosine signaling with indicators of dopamine metabolism dysfunction in SZ pathology. Specifically, it is hypothesized that adenosine signaling deficits will correlate with increased levels of phenylalanine, tyrosine and HVA in SZ patient plasma.

Table 2. Summary of findings in phenylalanine and tyrosine assays in schizophrenia

Author	Year	Samples	Findings	Implication	Limitation
Okusaga et al	2014	1000 SZs, 950 HCs	Phe increased in SZ plasma; Phe/Tyr ratio increased	Results suggest an abnormal PAH function caused by immune activation and oxidative stress. High Phe in plasma could result in intellectual disability in SZ.	Antipsychotic medication; samples are collected without fasting protocol
Poisner	1960	40 SZs, 15 HCs	Phe increased in SZ serum	High Phe level is related to mental illness.	Antipsychotic medication
Bjerkstedt et al	1985	37 SZs, 65 HCs	Phe increased in SZ plasma	The disruption of monoamine neurotransmitter synthesis is suggested.	Combined drug naïve group with medication group
Tortorella et al	2001	11 SZs, 11 HCs	Tyr increased in SZ plasma	Indicating that PAH activity may be inhibited in the early-onset patients with schizophrenia.	Small sample size; Antipsychotic medication
Rao et al	1992	77 SZs, 90 HCs	Phe increased in SZ plasma	As Tyr competes with tryptophan and Phe to get into the brain, findings may imply more Phe gets into the brain.	Antipsychotic medication

Abbreviations: Phe = phenylalanine; Tyr = tyrosine; SZs = schizophrenia patients; HCs = healthy control subjects.

1.6 DISSERTATION DESIGN

This dissertation is designed to test the adenosine hypothesis mentioned above (Lara et al. 2006, Boison et al. 2012) through an analytical approach using clinical samples. The central hypothesis is: Due to a purinergic signaling homeostatic imbalance, adenosine signaling deficits are present in SZ patients and correlate with clinical psychopathology in these

individuals with SZ. Specifically, it is proposed that adenosine signaling deficits will correlate with hyperdopaminergic signaling, increased membrane lipolysis and clinical symptomatology in SZ patients. Second, it is proposed that antipsychotic treatment will be associated with at least partial restoration or normalization of the adenosine signaling in SZ patients. This hypothesis would be supported by evidence for each of four specific aims:

Aim 1: Test if adenosine signaling deficits present in SZ patients. This aim will be addressed in Chapter 2.

Aim 2: Test if antipsychotics can affect and restore back adenosine signaling. This aim will be addressed in Chapter 3.

Aim 3: Test correlations of adenosine signaling deficit within purine pathway and with classic theories in SZ pathology. In this dissertation, we will test relationships between (A) adenosine signaling and lipid theory; (B) adenosine signaling and dopamine theory. This aim will be addressed in Chapters 4-6.

Aim 4: Test if adenosine signaling correlates with SZ symptomatology. This aim will be addressed in Chapter 7.

2.0 ADENOSINE SIGNALING DEFICITS IN SCHIZOPHRENIA PATIENTS

2.1 BACKGROUND

In this chapter, we tested if adenosine signaling deficits are present in SZ patients as hypothesized in the adenosine theory. Target analytes that we measured are adenosine, ADA and inosine. The ADA metabolizes adenosine to inosine in both CNS and peripheral tissues. Currently no research was performed measuring adenosine, ADA activity and inosine levels all together in SZ patients. However, we did find genetic studies showed lower frequency of the low activity ADA allelic variant in SZ patients (Dutra et al., 2010). It has also been reported that in chronic SZ patients, there is an increased serum ADA activity (Brunstein et al., 2007). These findings implied that an increased ADA activity might also be found in our study at least in peripheral tissues for the SZ group.

This chapter also serves as the aim 1 of the dissertation. This aim is further supported by three sub-aims. Results & discussion are based on the flow of following sub-aims:

Aim 1a. Test if peripheral tissue levels of analytes in adenosine signaling can predict those levels in the CNS. Here, we checked correlations of each analyte in adenosine signaling between cerebrospinal fluid (CSF) levels and plasma levels. Both healthy control (HC) group and SZ group were involved in this experiment.

Aim 1b. Test if there is an illness effect on adenosine signaling. For this aim, we compared adenosine, inosine and ADA activity levels between chronic off-treatment SZ patient

group and HC group. Group information is illustrated in experimental procedure section. Comparisons were made both in CSF and in plasma. We also compared correlations of adenosine and its major metabolizing enzyme (ADA) between off-treatment SZ group and HC group.

Aim 1c. Test if adenosine signaling deficit is a trait-like feature of SZ. Traits here are representing characteristic alterations of SZ that are consistent and long lasting. Unlike traits, which are stable characteristics, states are temporary level changes that depend on a person's situation and treatments at a particular time. To test this aim, we compared adenosine, inosine and ADA activity levels among first-episode drug naive schizophrenia patient (FEANS) group, disease control (DC) group and HC group. We also compared correlations of adenosine and ADA among these three groups.

It is predicted that adenosine levels will be reduced in the SZ patients, and that this reduction will be associated with increased activity of its major metabolizing enzyme (ADA). Consequently, an increased product (inosine) level should be observed. Also, it is predicted that we will observe that the correlation between adenosine and ADA breaks down in SZ patients. Overall, we predict adenosine signaling deficits are present in SZ patients.

2.2 EXPERIMENTAL PROCEDURES

2.2.1 Clinical design

1. **Chronic SZ and control groups.** Twenty-four SZ patients were from clinical services of the VA Pittsburgh Healthcare System (VAPHS). All procedures were approved by the VAPHS Highland Drive Institutional Review Board. After providing informed consent for their participation, patients were recruited after having had at least two years of treatment on antipsychotics. Diagnostic assessments and clinical symptom ratings were performed by experienced research clinicians. Patients were on stable doses of antipsychotics and voluntarily withdrawn from the treatment for two month. Both CSF and plasma samples were collected two months after the antipsychotic withdrawal date. In addition, 10 age-, race-, BMI- and gender-matched HC subjects were also recruited for comparison. The demographic table of subjects is shown below (Table 3).

Table 3. Demographic table of recruited subjects in chronic SZ and control groups

	HC	Off-treatment
Numbers	10	24
Gender ^a	6/4	15/9
Age	45.4 \pm 7.8	46.9 \pm 8.9
BMI	26.7 \pm 5.2	28.0 \pm 6.6
Race ^b	7/3/0/0	16/7/1/0

^a Male/Female. ^b Caucasian / African American / Pacific Asian / others.

Abbreviations: HC = healthy controls; Off-treatment = chronic schizophrenia patients that are withdrawn from antipsychotics for two months; FEANS = first episode antipsychotic naïve

schizophrenia patients. Note: age and BMI data are expressed in mean \pm standard deviation.

2. FEANS and control groups. Forty SZ patients were recruited during their first episode of psychosis after they provisionally met Diagnostic and Statistical Manual of mental disorders (DSM)-IV criteria for schizophrenia, schizophreniform, or schizoaffective disorder based on the Structured Clinical Interview for DSM Disorders (SCID). Diagnostic assessments and clinical symptom ratings were performed by experienced research clinicians. Initial and follow-up diagnoses were confirmed at diagnostic conferences attended by research faculty and staff, and chaired by an experienced psychiatrist. Blood samples were collected and clinical symptoms were evaluated prior to initiation of clinicians' choice of antipsychotic agents. In addition, 52 age-, race-, BMI- and gender-matched HC subjects and 24 patients with other psychiatric disorders (including bipolar disorder and major depression) were also recruited for comparison. The demographic table of subjects is shown below (Table 4).

Table 4. Demographic table of recruited subjects in FEANS and control groups

	HC	DC	FEANS
Numbers	52	24	40
Gender ^a	31/20	13/11	26/14
Age	26.3 \pm 7.4	26.9 \pm 9.9	22.7 \pm 8.1
BMI	25.2 \pm 4.4	25.2 \pm 6.9	23.3 \pm 5.3
Race ^b	34/10/3/4	17/5/1/1	20/16/2/2

^a Male/Female. ^b Caucasian / African American / Pacific Asian / others.

Abbreviations: HC = healthy control subjects; DC = disease controls; FEANS = first episode antipsychotic naïve schizophrenia patients. Note: age and BMI data are expressed in mean \pm

standard deviation; in HC group, one subject is missing sex and race information. Three subjects in FEANS group and one in DC group are missing BMI data due to missing height/weight values.

2.2.2 Sample preparation

CSF and blood samples were collected from subjects mentioned above. None of the subjects were currently on anticoagulants. Plasma aliquots were collected from blood samples after centrifugation, and then transferred to 2 mL tubes. All samples were stored in -80°C freezers. QC (quality control) samples were made from the sample pools in each group, respectively. All samples used in this dissertation went through these preparation procedures.

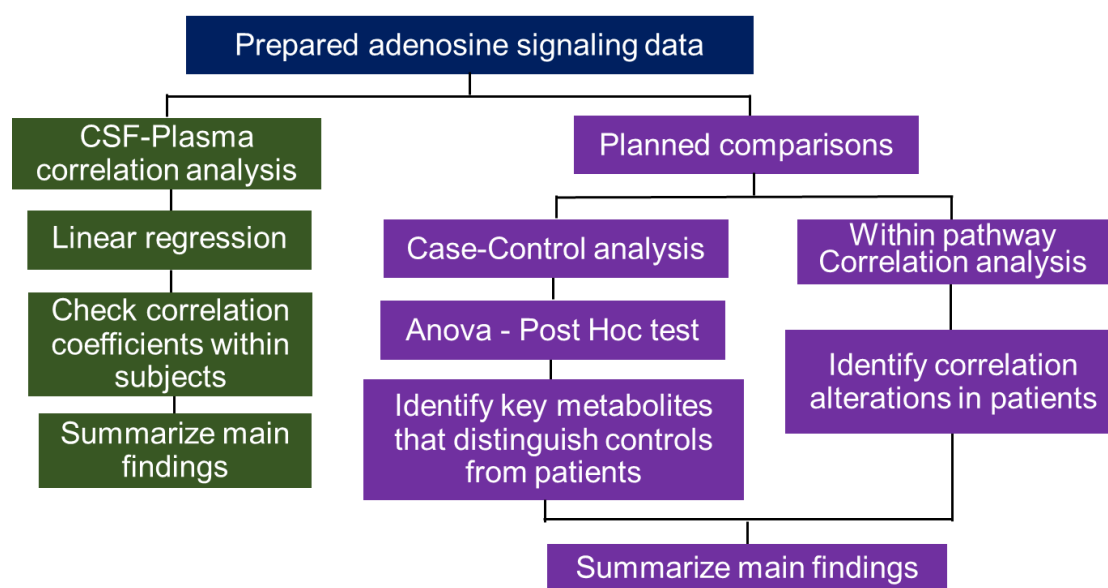
2.2.3 Enzyme-linked immunosorbent assay

Adenosine and inosine levels were assayed by using enzyme-linked immunosorbent assay (ELISA) kits from Biovision (K327 & K712). For adenosine assay kit, linear standard curve range is from 0.01-0.5 μM . The Lower Limit of Quantification is 0.0025 μM . Intra assay percentage of CV is below 2.0% and inter assay percentage of CV is below 3.0%. For inosine assay kit, linear standard curve range is from 0.1-5 μM . The Lower Limit of Quantification is 0.04 μM . Intra assay percentage of CV is below 2.0% and inter assay percentage of CV is below 2.5%. ADA activity levels were assayed by using ELISA kits from Diazyme (DZ117A-K). For ADA assay kit, linear standard curve range is from 0-200 I/U. The Lower Limit of Quantification is 0.075 I/U. Intra assay percentage of CV is below 4.5% and inter assay

percentage of CV is below 5.0%. Each assay was conducted by strictly following the protocol that was recommended by the kit. Readings were obtained from a PerkinElmer micro plate reader (Wallac 1420). Adenosine and inosine data were read at Ex/Em = 535/587 nm (fluorometric method) while ADA activity data were read at 550 nm (colourimetric method). Each sample was tested in duplicates and QCs were used to check the differences between plates.

2.2.4 Data analysis

Data analysis for this chapter followed the flow chart below (Figure 4). In brief, data in VA chronic SZ & control groups were used for correlation analyses between CSF and plasma. For each single analyte, each subject in this group had matched CSF and plasma data; therefore, correlations could be estimated within subjects (i.e. each subject served as its own control). For the comparisons between groups, analysis of variance (ANOVA) tests were used to test differences in analyte levels across HCs, DCs and FEANS. Follow-up post-hoc tests were



corrected by Bonferroni corrections. T-tests were used to compare the chronic off-treatment group and the HC group. For comparison of correlations of adenosine and ADA between groups, Pearson correlation coefficients were used to test the association. Fisher's r to z transformation was applied and correlation coefficients of adenosine vs ADA among different groups were compared. All data and results were checked for skewness by quantile-quantile plot. All tests were two-tailed.

2.3 RESULTS

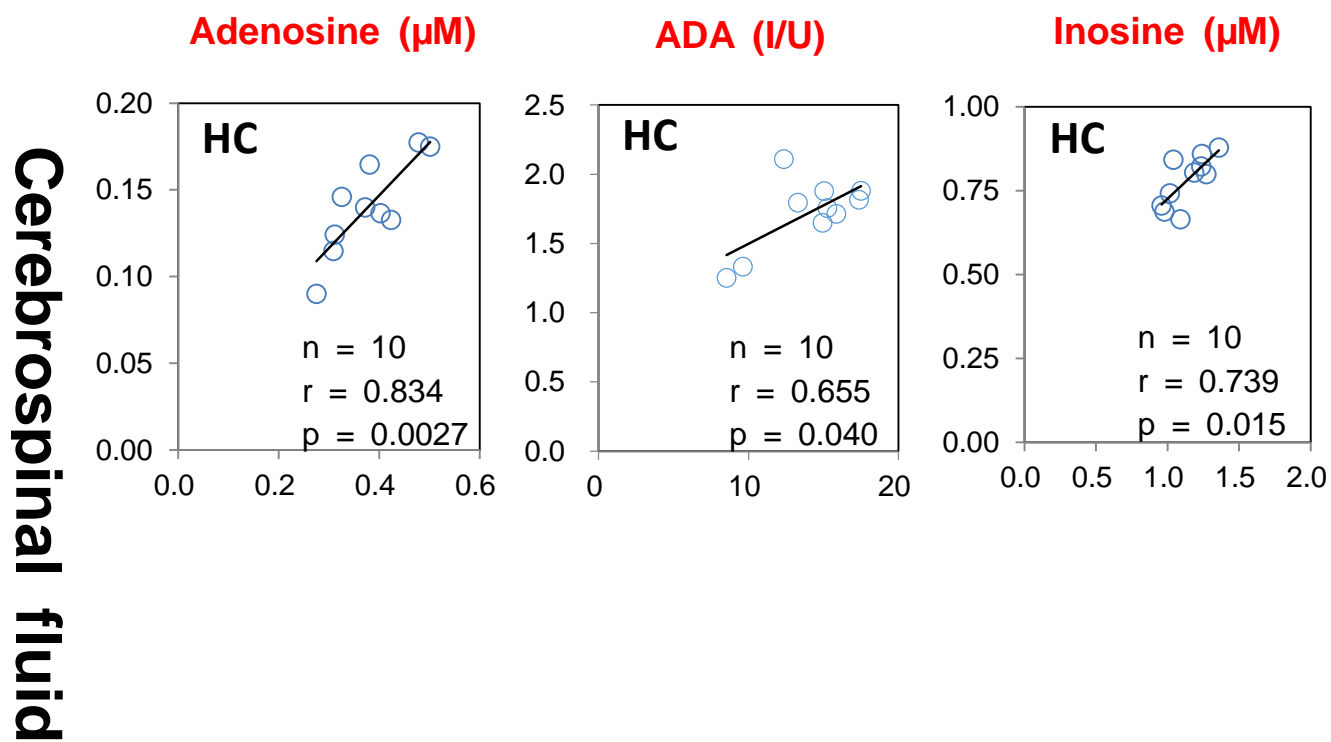
2.3.1 Correlations between CSF and plasma analyte levels within subjects

Consistent with expectation, significant correlations ($p < 0.05$) were observed between CSF

Figure 4. Data analysis flow chart for chapter 2. and plasma analyte levels in most cases (Figure 5). The correlation of

CSF with plasma ADA levels in the SZ group only showed a trend for significance ($p < 0.1$).

Also, with the exception of the CSF/plasma association for ADA levels in SZ, significant correlations were found for both HC and SZ groups.



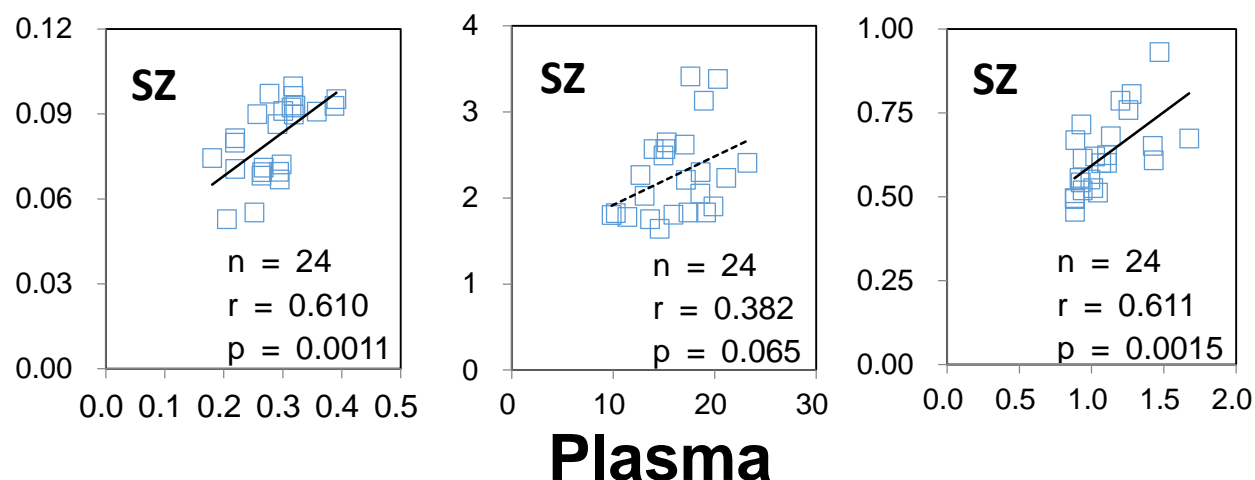


Figure 5. Correlations between CSF and plasma levels of adenosine, ADA activity and inosine in HC and chronic SZ groups.

Abbreviations: ADA = adenosine deaminase; HC = healthy controls; SZ = schizophrenia patients.

2.3.2 Comparison of CSF analytes' levels between HC group and chronic SZ off-treatment group

In CSF samples, significant reduction of adenosine and inosine levels were observed in off-treatment SZ patients in comparison with HCs. As expected, a significant increase of ADA activity levels was observed in off-treatment SZ patients when compared with HCs (Figure 6).

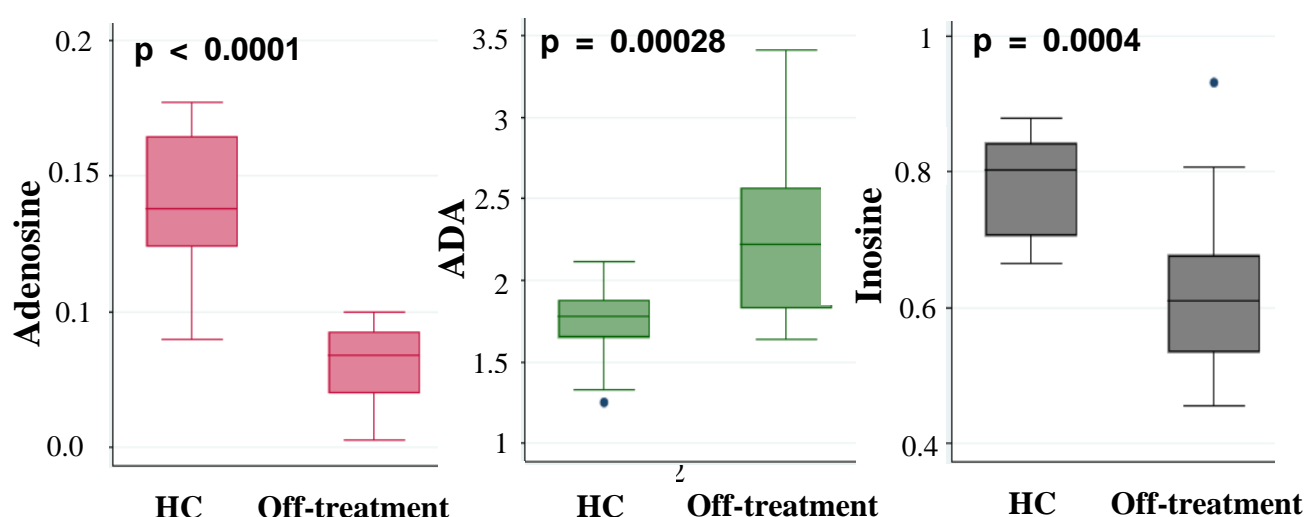
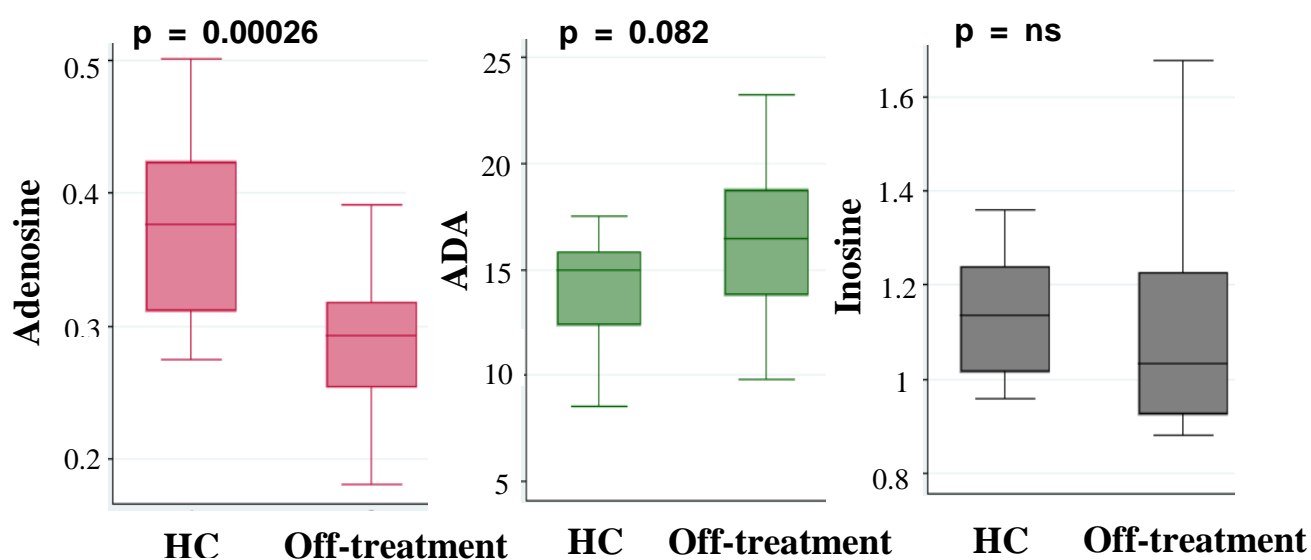


Figure 6. Comparisons of CSF adenosine, ADA activity and inosine levels in HC group and SZ off-treatment group . ADA = adenosine deaminase; HC = healthy controls. Units: adenosine and inosine are in μM , ADA is in I/U.

2.3.3 Comparison of plasma analytes' levels between HC group and chronic SZ off-treatment group

We then compared these analytes' levels between HC group and chronic SZ off-treatment group. In plasma samples, a significant reduction levels of adenosine was also observed in off-treatment SZ patients. However, no significant change was found between the off-treatment group and the HC group with regard to ADA activity and inosine levels (Figure 7).



2.3.4 Correlations between adenosine and ADA in HC group and SZ off-treatment group

As expected, significant inverse correlations were observed between adenosine and ADA in HC group (Figure 8). In contrast, a highly disrupted correlation between adenosine and ADA

Figure 7. Comparisons of plasma adenosine, ADA activity and inosine levels in HC group and SZ off-treatment group.

Abbreviations: ADA = adenosine deaminase; HC = healthy controls. Units: adenosine and inosine are in μM , ADA is in I/U.

adenosine with ADA shown in the HC group, there was a trend ($p < 0.07$) for a positive correlation of adenosine with ADA in the off-treatment SZ group on CSF measures. No significant correlation was observed between adenosine and ADA in the off-treatment SZ group on the plasma measures (Figure 8). Fisher's z test showed a significant difference of correlation coefficients between HC and off-treatment groups in the CSF samples ($z = 2.94$, $p = 0.0033$) but not in the plasma samples ($z = 1.94$, $p = 0.0524$).

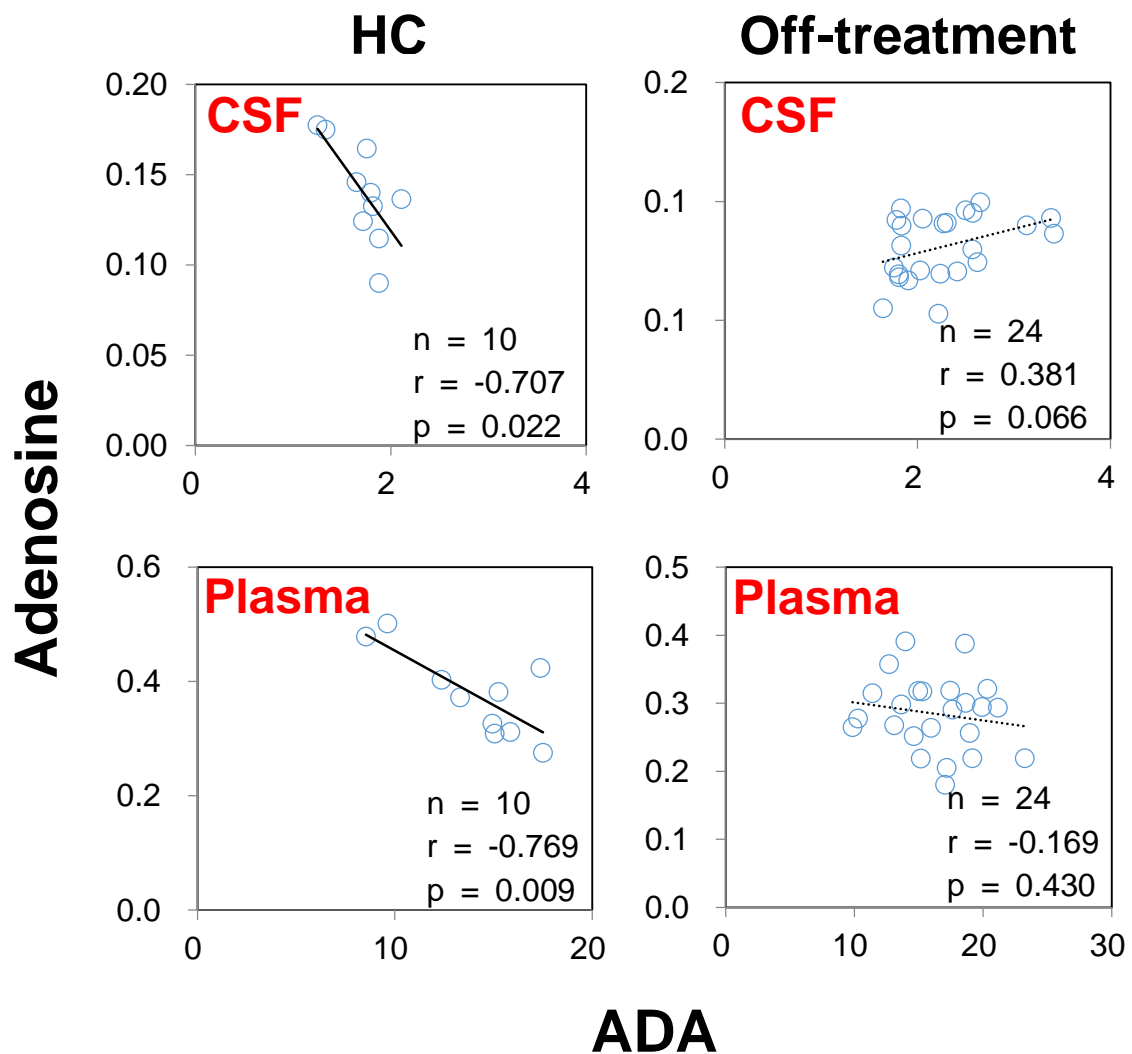


Figure 8. Correlations between adenosine and ADA in HC group and in off-treatment SZ group.

Abbreviations: ADA = adenosine deaminase; HC = healthy controls; CSF = cerebrospinal fluid. Units: adenosine and inosine are in μM , ADA is in I/U.

2.3.5 Comparisons of plasma analyte levels among HC group, DC group and FEANS group

As with the findings in 2.3.3, significantly lower adenosine plasma levels were observed in the FEANS group when compared with the HC and DC groups. However, no significant changes

of ADA activity and inosine levels were observed among these three groups (Figure 9).

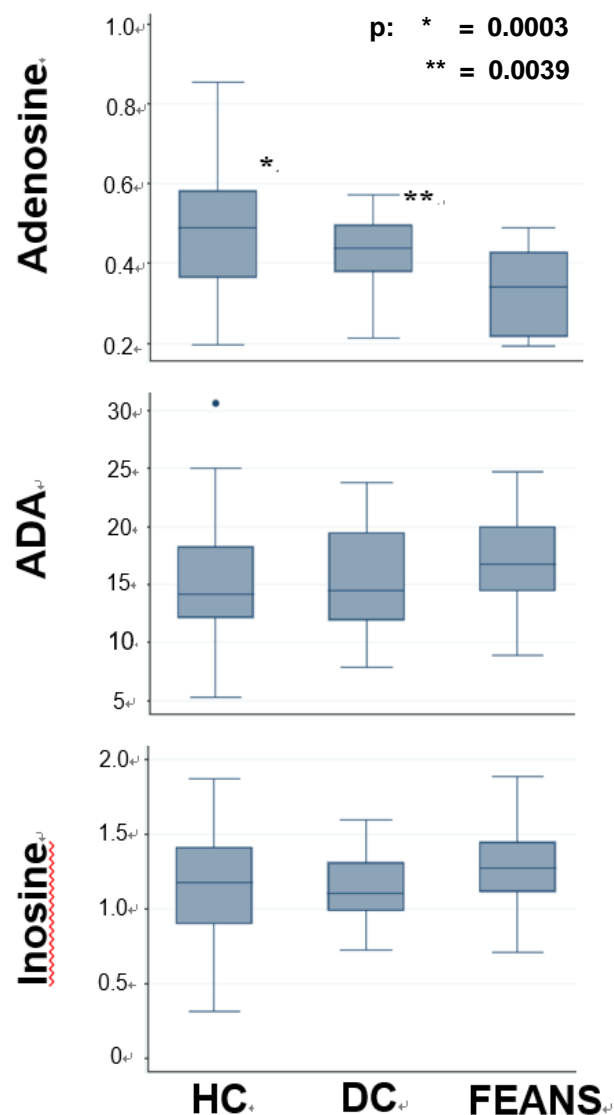


Figure 9. Comparisons of plasma analytes' levels among HC, DC and FEANS groups.
Abbreviations: ADA = adenosine deaminase; HC = healthy controls; DC = disease controls.
Units: adenosine and inosine are in μ M, ADA is in I/U.

2.3.6 Correlations between adenosine and ADA among HC, DC and FEANS groups

Finally, we tested correlations between adenosine and ADA among the HC, DC and FEANS groups. Significantly inversed correlations of adenosine with ADA in the HC and DC groups were observed. In contrast, no significant correlations were observed between adenosine and

ADA for the FEANS group (Figure 10). However, Fisher's z test did not show significant difference of correlation coefficients between HC and FEANS groups ($z = -0.58$, $p = 0.5619$) or DC and FEANS groups ($z = -1.72$, $p = 0.0854$).

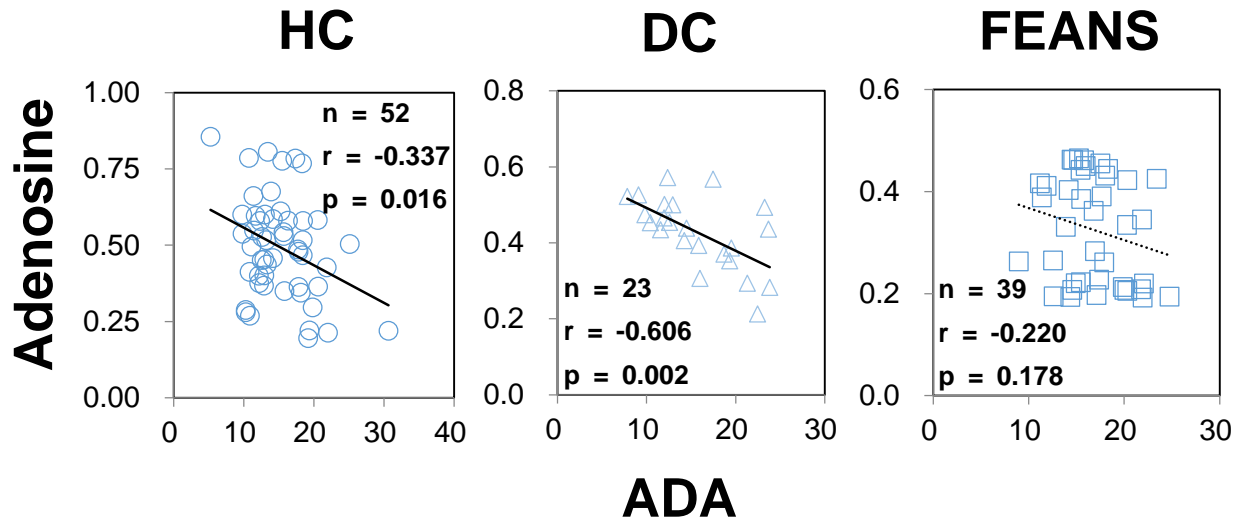


Figure 10. Correlations between adenosine and ADA among HC, DC and FEANS groups. Abbreviations: ADA = adenosine deaminase; HC = healthy controls; CSF = cerebrospinal fluid. Unit: adenosine and inosine are in μM , ADA is in I/U.

2.4 DISCUSSION

From results in 2.3.1 we observed significant correlations (with a single exception, i.e. and association at a trend level) between CSF and plasma analyte levels in both HC group and SZ group. This finding would suggest that peripheral tissue adenosine, ADA activity and inosine levels can serve as indicators of CNS adenosine, ADA activity and inosine levels. One previous study assayed the ADA activity in CSF and in plasma simultaneously in order to help to diagnose tuberculous meningitis and they found both absolute CSF ADA activity levels and

the ratio of CSF to plasma ADA activity levels could distinguish the disease group from the control group (Donald et al. 1986). To our knowledge, no other study has systematically correlated adenosine signaling in CNS with that in the peripheral tissues.

As predicted, significantly lower adenosine levels and higher ADA activity levels in the CSF of chronic off-treatment SZ patients were observed. These findings suggested that there is an illness effect on adenosine signaling in the CNS in SZ patients compared with HC subjects. Reduction of adenosine levels may result from increased metabolizing enzyme (ADA) activity, suggesting an increased breakdown of adenosine by ADA. However, the observation of significantly lower metabolite (inosine) levels is not consistent with the prediction. An increased breakdown of adenosine should have yielded more inosine, yet such increase was not observed. We hypothesized that it may be due to an increased activity of nucleoside phosphorylase and increased production of hypoxanthine and the following metabolites on the purine pathway. This hypothesis was tested in Chapter 4. Significantly lower adenosine levels in plasma indicated that the illness effect on adenosine signaling in CNS can also be found in peripheral tissues. The unchanged plasma inosine levels may also be due to the increased activity of nucleoside phosphorylase and increased production of hypoxanthine and the following metabolites on purine pathway. With regard to ADA activity, there are two possible reasons for failure to observe a significant change in plasma ADA activity in the chronic off-treatment SZ group. First, it may be due to the limitation of sample size ($n = 24$), given that we observed a trend for higher ADA levels in the off-treatment SZ group as compared with the HC group ($p = 0.082$). Secondly, it may imply that peripheral measures are not as sensitive to

an illness effect on ADA activity as are CSF levels. Further studies are required to investigate CNS vs peripheral tissue ADA activity differences as well as to explore possible underlying mechanism (s).

For the correlation between adenosine and ADA, we found that both in CSF and plasma samples, adenosine levels inversely correlated with ADA levels in control subjects. This finding is consistent with our expectation, as ADA is the major metabolizing enzyme of adenosine. The significant correlation revealed that in normal condition, enzyme (ADA) activity is tightly connected to the precursor (adenosine). However, in SZ patients, we found evidence for highly disrupted connections between adenosine and ADA. Whereas there were significant inverse ADA/adenosine correlations for the HC group in both CSF and plasma, there was a trend for a positive correlation in the CSF of the chronic off-treatment SZ group. Furthermore, there was no significant plasma ADA/adenosine correlation for the chronic off-treatment SZ group. As is illustrated in the introduction section, the reaction from adenosine to inosine via ADA might be substrate-limited, which explained the lack of adenosine-ADA association by the reduced levels of substrates. Therefore, it is suggested that adenosine signaling is disrupted by SZ in both CNS and peripheral tissues.

Significantly lower plasma adenosine levels in the FEANS group were observed and suggested that the potential adenosine signaling deficits may exist at the early course of the disease. Compared with findings in section 2.3.2 & 2.3.3, no significant differences in analyte levels were observed between the chronic off-treatment SZ group and the FEANS group. This suggested that the exposure & 'left-over' effect of the treatment did not play a significant role

here. Thus, these findings would suggest that CSF adenosine and ADA levels may serve as potential biomarkers of SZ and that these deficits may be trait-like features of SZ.

It is important to note that in both the HC and DC groups, adenosine levels inversely correlated with ADA levels – a finding that is consistent with results in 2.3.4. This finding supports the notion that in the normal condition, enzyme (ADA) activity is tightly connected to the precursor (adenosine). We also observed a disrupted correlation between adenosine and ADA in the FEANS group; however, this disturbance was not as severe as that observed in chronic off-treatment SZ group as being supported by Fisher's z test results. These observations imply that adenosine signaling may start to get disrupted early in the course of the disease and that such alteration exacerbates as the disease progresses.

2.5 CONCLUSIONS

1. Within subjects, significant correlations were observed in most cases between CSF and plasma analyte levels in both SZ group and HC group. These significant correlations indicated that peripheral tissue levels of adenosine signaling indices could serve as important indicators of adenosine signaling in the CNS.
2. Significant decreased adenosine levels and significant increased ADA activity levels were revealed both in chronic off-treatment SZ group and FEANS group when compared with control groups, which suggested that there might be an adenosine hypofunction in the schizophrenia pathology. According to these findings, adenosine levels in CSF & plasma and

ADA activity levels in CSF may serve as potential biomarkers for SZ.

3. Significantly reduced CSF levels and insignificantly changed plasma levels of inosine are not coherent with our original prediction. We think it might be caused by increased activity of nucleoside phosphorylase and increased production of hypoxanthine & following metabolites in SZ pathology.

4. Significant correlations between precursor (adenosine) and its major metabolizing enzyme (ADA) were observed in control groups, indicating tight connections between adenosine and ADA under normal condition. However, such tight connections were lost in SZ patients. Insignificant correlations were observed in both FEANS group and chronic off-treatment SZ group, implying the disruption of the adenosine signaling by SZ. Interestingly, a more disrupted correlation between adenosine and ADA were found in chronic off-treatment SZ group as compared with that in FEANS group, which implied that the disturbance of adenosine signaling begins at the early course of disease and exacerbates as disease progresses.

Overall, in this chapter, alterations of levels and breakdown of connections between analytes in adenosine signaling were observed and adenosine signaling deficits were suggested in SZ patients. Such deficits tend to be trait-like features of SZ according to the data in this chapter.

3.0 TREATMENT EFFECT ON ADENOSINE SIGNALING

3.1 BACKGROUND

In this chapter, we tested if adenosine signaling can be affected by antipsychotics. As mentioned in the introduction section, activation of adenosine receptors would have inhibitory effect on DRs. Adenosine agonists produce similar pharmacological effects as dopamine antagonists (Ferré 1997). Therefore, we tested if dopamine blocker could restore adenosine signaling by inhibiting dopaminergic signaling. Antipsychotics used for this study are haloperidol and risperidone which belong to first- and second-generation antipsychotics, respectively. The mechanism of action of most first- and second-generation antipsychotics is post-synaptic blockade of brain D₂ receptors (Jibson. 2016). Most second-generation antipsychotics differ from first generations pharmacologically in that they also bind to 5-HT₂ receptors. Since interactions between serotonin receptors and ARs are still not clear at current stage, it would be interesting to explore the potential differences between first- and second-generation antipsychotic effects on adenosine, ADA, and inosine levels in SZ patients.

This chapter also serves as the aim 2 of the dissertation. This aim is further supported by two sub-aims. Results & discussion are based on following sub-aims:

Aim 2a. Test if antipsychotics can affect adenosine signaling. Test if the treatment effect were different between CNS and peripheral tissues. Here we were using chronic on-treatment SZ patient CSF & plasma samples. We made comparisons between on-treatment SZ

group and off-treatment SZ group within subjects. HC group data that were collected from Chapter 2 were also used as reference.

Aim 2b. Test short- vs long-term treatment effect in longitudinal study. To test this aim, we compared adenosine, inosine and ADA levels among antipsychotic naïve baseline and follow-up time points (1M, 6M and 12M) after treatments. Moreover, we also compared correlations between precursor (Adenosine) and its major metabolizing enzyme (ADA) in baseline and all time points.

We were predicting that antipsychotics can affect and restore back adenosine signaling. Specifically, we predicted that treatment will increase levels of adenosine and inosine. Also, ADA levels will be reduced back to normal levels. The broken correlations between adenosine and ADA in the baseline could be normalized (or partially normalized) after the treatment.

3.2 EXPERIMENTAL PROCEDURES

3.2.1 Clinical design

1. **Chronic SZ and control groups.** Twenty-four SZ patients were recruited from clinical services of the VA Pittsburgh Healthcare System (VAPHS). All procedures were approved by the VAPHS Highland Drive Institutional Review Board. After providing informed consent for their participation, patients were recruited after having had at least two years of treatment on antipsychotics. Diagnostic assessments and clinical symptom ratings were performed by

experienced research clinicians. Patients were on stable doses of antipsychotics and voluntarily withdrawn from the treatment for two month. Both CSF and plasma samples were collected before and two months after the antipsychotic withdrawal date. The demographic information of on-treatment SZ group has been shown in table 3. In addition, 10 age-, race-, BMI- and gender-matched HC subjects were also recruited for comparison.

2. FEANS baseline, follow-up time points and control groups. Forty SZ patients were recruited during their first episode of psychosis after they provisionally met Diagnostic and Statistical Manual of mental disorders (DSM)-IV criteria for schizophrenia, schizophreniform, or schizoaffective disorder based on the Structured Clinical Interview for DSM Disorders (SCID). Diagnostic assessments and clinical symptom ratings were performed by experienced research clinicians. Initial and follow-up diagnoses were confirmed at diagnostic conferences attended by research faculty and staff, and chaired by an experienced psychiatrist. Blood samples were collected and clinical symptoms were evaluated prior to initiation of clinicians' choice of antipsychotic agents. Follow-up blood samples were collected after 1 month, 6 months and 12 months treatment by risperidone. Due to the funding limitations, there are some drop outs of base line subjects in those follow-up groups. The demographic table of subjects is shown below (Table 5). In addition, 52 age-, race-, BMI- and gender-matched HC subjects were also recruited for comparison.

Table 5. Demographic table in longitudinal study

	HC	Baseline	1M	6M	12M
Numbers	52	40	28	27	17
Gender ^a	31/20	26/14	18/10	17/10	9/8
Age	26.3 \pm 7.4	22.7 \pm 8.1	23.0 \pm 8.0	23.2 \pm 8.4	23.5 \pm 8.5
BMI	25.2 \pm 4.4	23.3 \pm 5.3	23.2 \pm 6.0	23.2 \pm 6.0	23.0 \pm 6.4
Race ^b	34/10/3/4	20/16/2/2	15/10/2/1	15/9/2/1	9/5/2/1

^a Male/Female. ^b Caucasian / African American / Pacific Asian / others.

Abbreviations: HC, healthy control. Note: age and BMI data are expressed in means \pm standard deviations; in HC group, one subject is missing sex and race information. Three subjects in baseline, 1 month (1M) and 6 months (6M) groups and one subject in 12 months (12M) group are missing BMI data due to missing height/weight values.

3.2.2 Enzyme-linked immunosorbent assay

The ELISA assay experimental design has been illustrated in section 2.2.3. We used same commercial ELISA kits as mentioned in section 2.2.3. In this chapter, adenosine, inosine and ADA levels were measured in chronic on-treatment and first-episode post-treatment groups. Data collected from chronic off-treatment, FEANS and control groups in Chapter 2 were used for comparisons and reference.

3.2.3 Data analysis

1. **General analysis procedures.** Data analysis for this chapter followed the flow chart below (Figure 11). In brief, planned comparisons of analytes' levels were made between on- and off-treatment SZ groups via paired-t test. Correlations of adenosine and ADA were also analyzed in on- and off-treatment SZ groups via linear regressions. Fisher's r to z transformation was applied and correlation coefficients of adenosine vs ADA among different groups were compared. On the other hand, comparisons of adenosine, inosine and ADA levels between short- and long-term treatment groups were made by mixed linear model analysis. All raw data and results were checked for distribution via quantile-quantile plots and main findings were summarized thereafter. All tests were two-tailed.

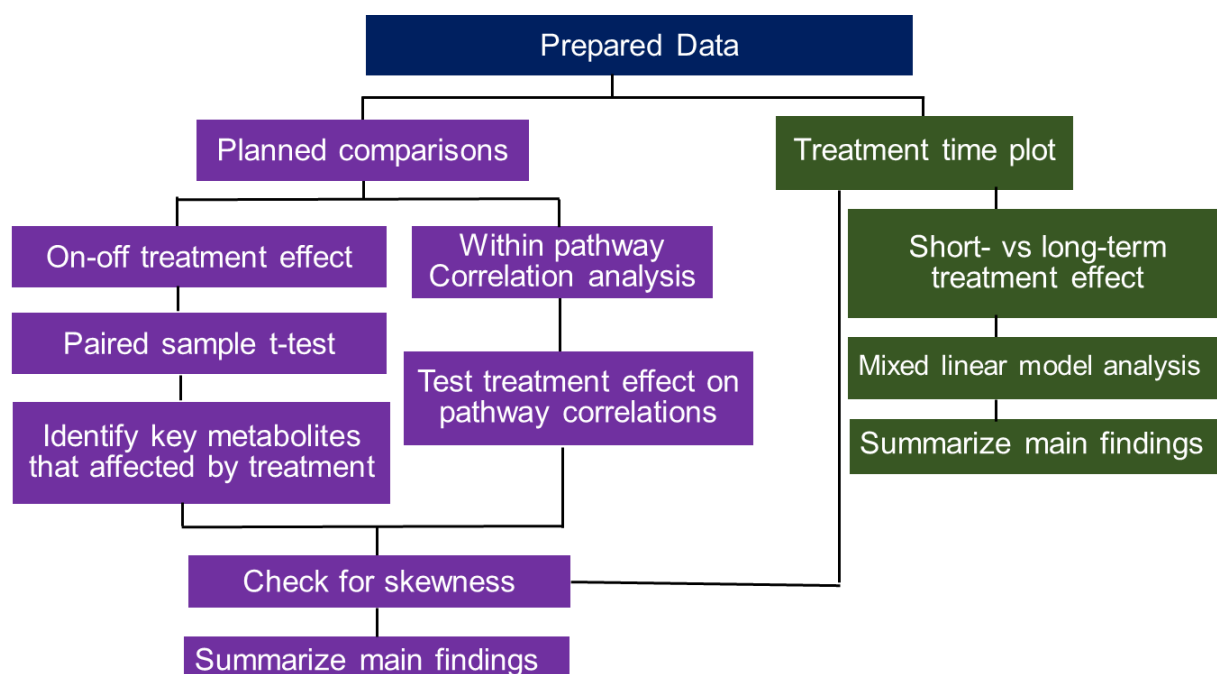


Figure 11. Data analysis flow chart for chapter 3.

2. **Mixed linear model analysis procedures.** Due to the differences of sample sizes and

covariates (Table 5) in the longitudinal study, mixed linear model might be a better option in this study. Here, three steps are shown below for mixed linear model analysis.

1) **Model structure selection.** In linear mixed model, adenosine level was set as dependent variable and no covariate was included at this step. Look for the lowest schwarz's bayesian criterion (BIC) among covariance structures among variance components, toeplitz, autoregressive and unstructured models. BIC is a criterion for model selection among a finite set of models, and the model with the lowest BIC is preferred (Bhat and Kumar. 2010).

2) **Test if ADA can serve as the mediator in the conversion from adenosine to inosine.**

- i. Test if covariates could affect adenosine levels. Set adenosine as the dependent variable. Set age, BMI, race, and sex as covariates. Test fixed effects for significance.
- ii. Test if adenosine levels can determine ADA's level. Run this step for two times. For the first time, set ADA as dependent variable and find out which covariate (s) could potentially serve as cofounder. For the second time, only include adenosine and potential cofounder (s) identified in first time as covariates. Test fixed effect for significance.
- iii. Test if ADA's level can determine inosine levels. Run this step for two times. For the first time, set inosine as dependent variable and find out which covariate (s) could potentially serve as cofounder. For the second time, only include ADA and potential cofounder (s) identified in first time as covariates. Test fixed effect for significance.

- iv. Test if adenosine level can determine inosine levels directly. Run this step for two times. For the first time, set inosine as dependent variable and find out which covariate (s) could potentially serve as cofounder. For the second time, only include adenosine and potential cofounder (s) identified in first time as covariates. Test fixed effect for significance.
- v. Test if adenosine & ADA levels can determine inosine's level. Run this step for two times. For the first time, set adenosine and ADA as dependent variables and find out which covariate (s) could potentially serve as cofounder. For the second time, only include adenosine, ADA and potential cofounder (s) identified in first time as covariates. Test fixed effect for significance.

3) Test the difference of analytes' levels among repeated measurements. Here we tested if adenosine, ADA and inosine levels were significantly changed among BL, 1M, 6M and 12M groups. Covariate (s) that showed significant ($p < 0.05$) levels in step 2) fixed effect tests were also included in this step as fixed effect covariates.

- i. Test if time would have effect on adenosine, ADA and inosine levels respectively. Each analyte level was set as dependent variable. Time intercept was set as random effect covariate and time was set as fixed effect covariate. Test fixed effect for significance.
- ii. Test if adenosine levels can predict inosine levels over time. Inosine levels was set as dependent variable. Time and adenosine were set as fixed effect covariates and time intercept was set as random effect. Test fixed effect for significance.

- iii. Test if ADA levels can predict inosine levels over time. ADA levels was set as dependent variable. Time and ADA were set as fixed effect covariates and time intercept was set as random effect covariate. Test fixed effect for significance.
- iv. Test if adenosine levels can predict ADA levels over time. ADA levels was set as dependent variable. Time and adenosine were set as fixed effect covariates and time intercept was set as random effect covariate. Test fixed effect for significance.
- v. Test if adenosine & ADA levels can predict inosine levels over time. Inosine levels was set as dependent variable. Time, adenosine and ADA levels were set as fixed effect covariates and time intercept was set as random effect covariate. Test fixed effect for significance.

3.3 RESULTS

3.3.1 Comparison of CSF analytes' levels between on- and off-treatment SZ group

As hypothesized, in CSF samples, significantly higher adenosine and ADA levels and significantly lower inosine levels were observed in on-treatment SZ group when comparing with those in off-treatment SZ group (Figure 12). Also, significantly lower adenosine levels in on-treatment SZ group were observed when comparing with those in HC group. Interestingly, no significant differences of ADA and inosine levels were found between on-treatment SZ group and HC group (Figure 12).

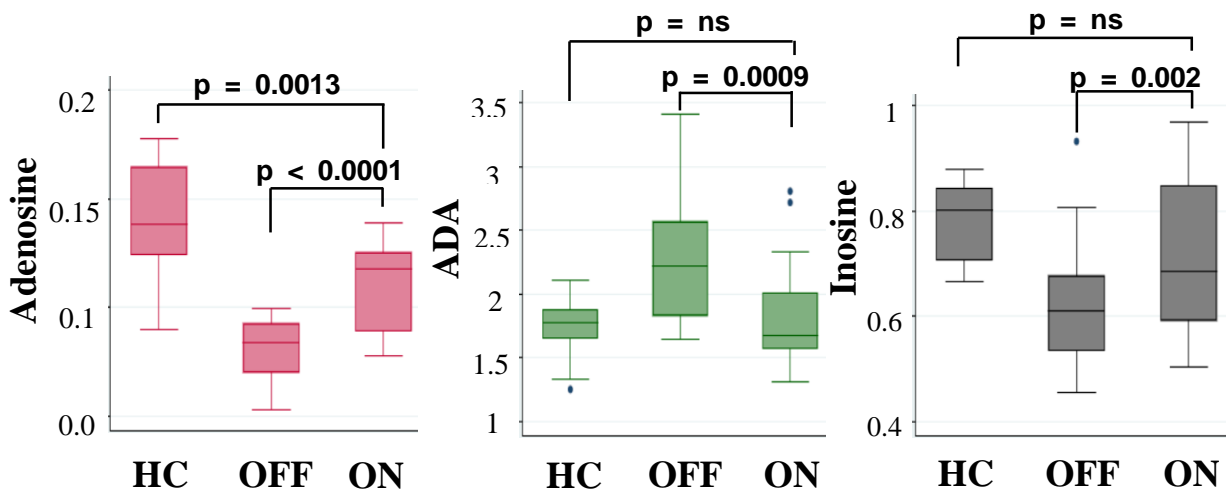


Figure 12. Comparisons of CSF adenosine, ADA and inosine levels among HC, on- and off-treatment SZ groups.

Abbreviations: ADA = adenosine deaminase; HC = healthy control; OFF = off-treatment SZ group; ON = on-treatment SZ group. Units: adenosine and inosine are in μM , ADA is in I/U.

3.3.2 Comparison of plasma analytes' levels between on- and off-treatment SZ group

In consistent with our finding in 3.3.1, in plasma samples, adenosine levels were observed that significantly higher in on-treatment SZ group than those in off-treatment SZ group. However, adenosine levels in on-treatment SZ group were observed that significantly lower than those in HC group. On the other hand, no significant changes of ADA and inosine levels were found between on-treatment SZ and off-treatment SZ groups (Figure 13).

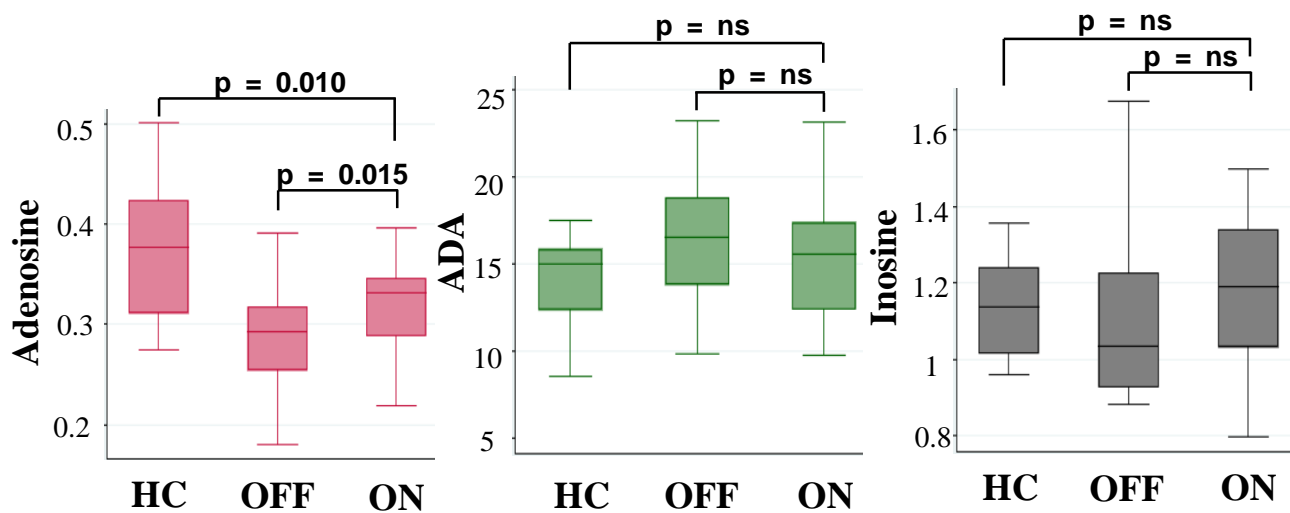


Figure 13. Comparisons of plasma adenosine, ADA activity and inosine levels among HC, on-treatment SZ and off-treatment SZ groups.

Abbreviations: ADA = adenosine deaminase; HC = healthy control; OFF = off-treatment SZ group; ON = on-treatment SZ group. Units: adenosine and inosine are in μM , ADA is in I/U.

3.3.3 Correlations of adenosine and ADA in on- and off-treatment SZ group

In general, we didn't observe significant correlations between adenosine and ADA in either on-treatment SZ group or off-treatment SZ group. Correlations of adenosine with ADA in

plasma showed a significant trend ($p = 0.081$) in on-treatment SZ group (Figure 14), but it should still be noted that this association is not significant ($p > 0.05$). Fisher's z test did not show significant difference of correlation coefficients between on- and off-treatment groups in either CSF samples ($z = -1.26$, $p = 0.2077$) or plasma samples ($z = -0.68$, $p = 0.4965$).

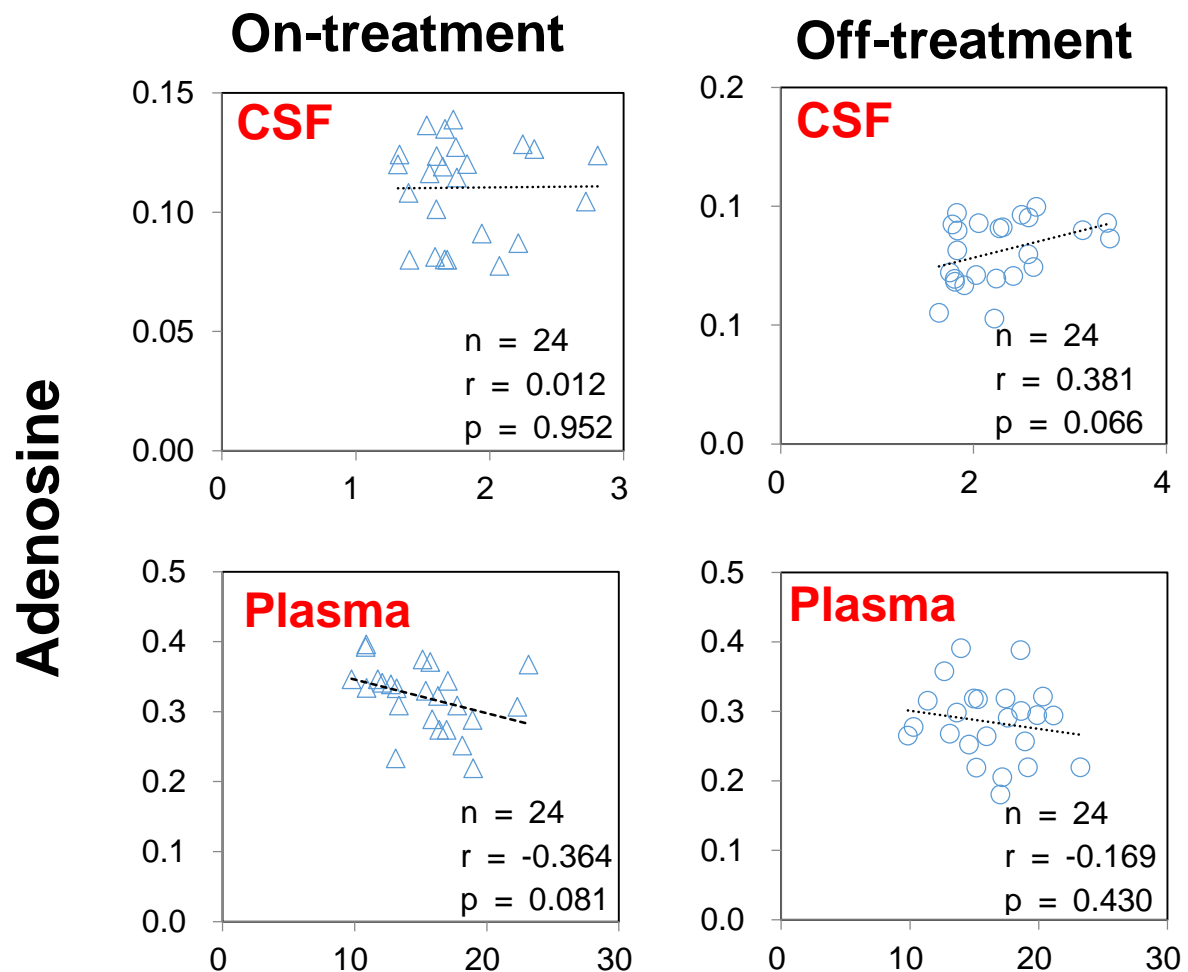


Figure 14. Correlations of adenosine and ADA in on- and off-treatment SZ groups.

Abbreviations: ADA = adenosine deaminase; CSF = cerebrospinal fluid. Units: adenosine and inosine are in μM , ADA is in I/U.

3.3.4 Mixed model analysis results in longitudinal study

1. Model structure selection. In this step, adenosine levels was set as dependent variable and no covariate or factor is included. Look for the lowest BIC among variance components, toeplitz, auto-regressive and unstructured models. Based on results showed below (Table 6), variance components structure was selected as a variance-covariance matrix.

Table 6. BIC values among different model structures

	VC	TP	AR (1)	UN
BIC	-128.0	-126.6	-126.6	-123.3

Abbreviations: BIC = schwarz's bayesian criterion; VC = variance components; TP = toeplitz; AR (1) = auto-regressive; UN = unstructured.

2. Test ADA's role as mediator in the conversion from adenosine to inosine. Five steps were then used to test ADA's role in this study using variance components model structure. Model setups and results were summarized in table 7. Results showed that ADA activity levels can be predicted by adenosine levels. However, inosine levels cannot be predicted by adenosine or ADA or adenosine & ADA levels. Therefore, ADA cannot serve as the mediator in the conversion from adenosine to inosine here. With regard to other covariates, we found that age and race could also affect results (i.e. age and race are significant covariates in this test).

Table 7. Test ADA as mediator in the conversion from adenosine to inosine

Model	First Run			Second Run		
	Dependent Variable	Covariates	Sig ^a	Dependent Variable	Covariates ^c	Sig ^a
1	ADE	ALL ^b	Age	NA	NA	NA
2	ADA	ADE, ALL	Race, ADE	ADA	Race, ADE	Race, ADE
3	INO	ADA, ALL	Race	INO	Race, ADA	Race
4	INO	ADE, ALL	Race	INO	Race, ADE	Race
5	INO	ADE, ADA, ALL	Race	INO	ADE, ADA, ALL	Race

^a Covariate (s) that showed significant ($p < 0.05$) levels in fixed effect tests. ^b Includes age, BMI, sex and race. ^c Include covariates that have shown significant ($p < 0.05$) levels in the first run. Abbreviations: ADE = adenosine; ADA = adenosine deaminase; INO = inosine; NA = not applicable.

3.3.5 Comparisons of analytes' levels among FEANS baseline, post-treatment and HC groups.

Here we used a mixed model approach to test if analytes' levels were significantly changed by treatments over time. As was shown below (Table 8), adenosine levels was observed that significantly changed between BL and all post-treatment groups. Significantly higher adenosine levels were observed in 1M, 6M and 12M groups when comparing with BL group (Figure 15). However, no significant change of adenosine levels were found among 1M, 6M and 12M groups. As for ADA activity and inosine levels, no significant changes were found among baseline and all post-treatment groups. When compared with healthy control group, no significant changes were observed in any post-treatment time point groups with regard to adenosine, ADA activity or inosine levels (Figure 15). Also, adenosine levels can predict ADA

levels over time by treatment while inosine cannot be predicted by adenosine, ADA or adenosine & ADA (Table 8). Within subjects, trajectories showed that over 90% of subjects have significantly higher adenosine levels in the 1M point when comparing with those in BL point. No significant trend of trajectories were observed from 1M point to 12M point. Also, no significant trend of trajectories were observed from BL to 12M point in terms of ADA activity and inosine levels (Figure 16).

Table 8. Mixed model analysis procedures and results

Model	Dependent Variable	Fixed effect covariates	Random effect covariate	Sig^a
1	ADE	Time ^b , Age, Race	Time intercept	Time
2	ADA	Time, Age, Race	Time intercept	None
3	INO	Time, Age, Race	Time intercept	None
4	INO	Time, Age, Race, ADE	Time intercept	None
5	INO	Time, Age, Race, ADA	Time intercept	None
6	ADA	Time, Age, Race, ADE	Time intercept	ADE
7	INO	Time, Age, Race, ADE, ADA	Time intercept	None

^a Covariate (s) that showed significant ($p < 0.05$) levels in fixed effect tests; ^b repeated measures from baseline to 12 months after the treatment. Abbreviations: ADE = adenosine; ADA = adenosine deaminase; INO = inosine.

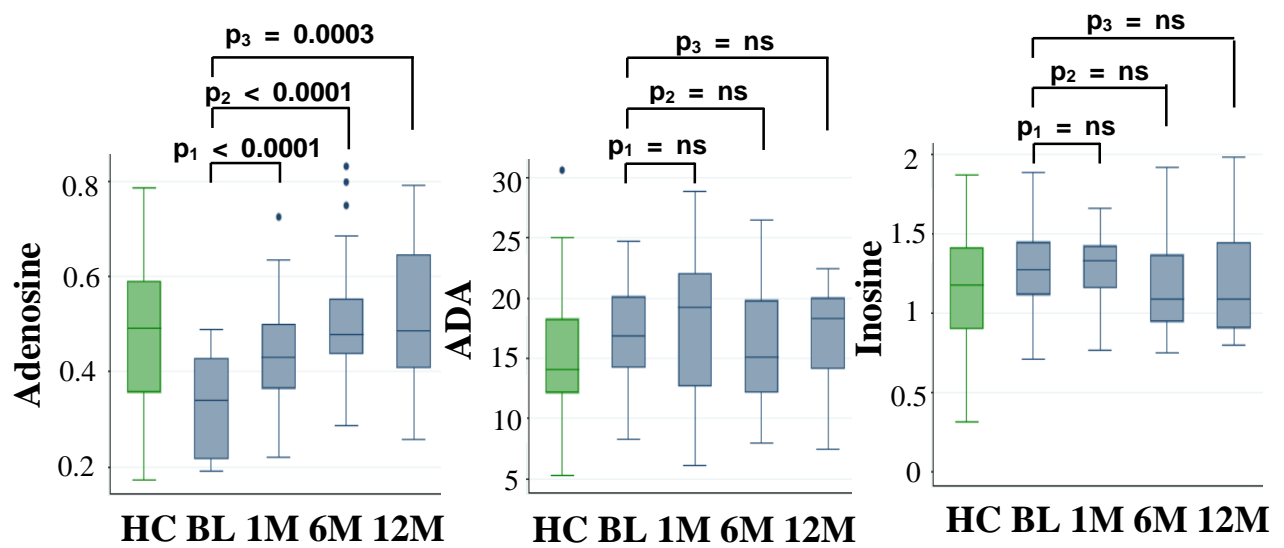
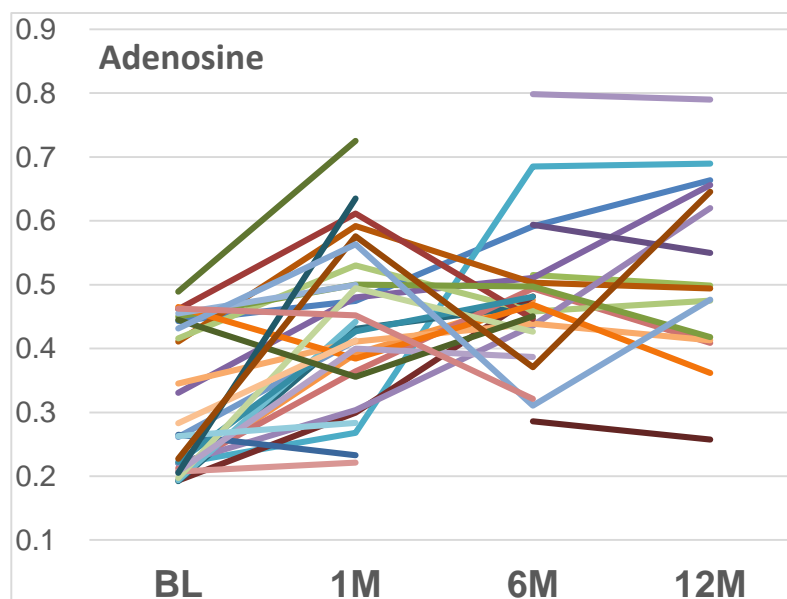


Figure 15. Comparisons of plasma adenosine, ADA activity and inosine levels in HC, baseline and post-treatment groups.

Abbreviations: ADA = adenosine deaminase; HC = healthy control; BL = baseline; 1M = 1 month after baseline; 6M = 6 months after baseline; 12M = 12 months after baseline. Units: adenosine and inosine levels are in μM , ADA levels are in I/U.

a



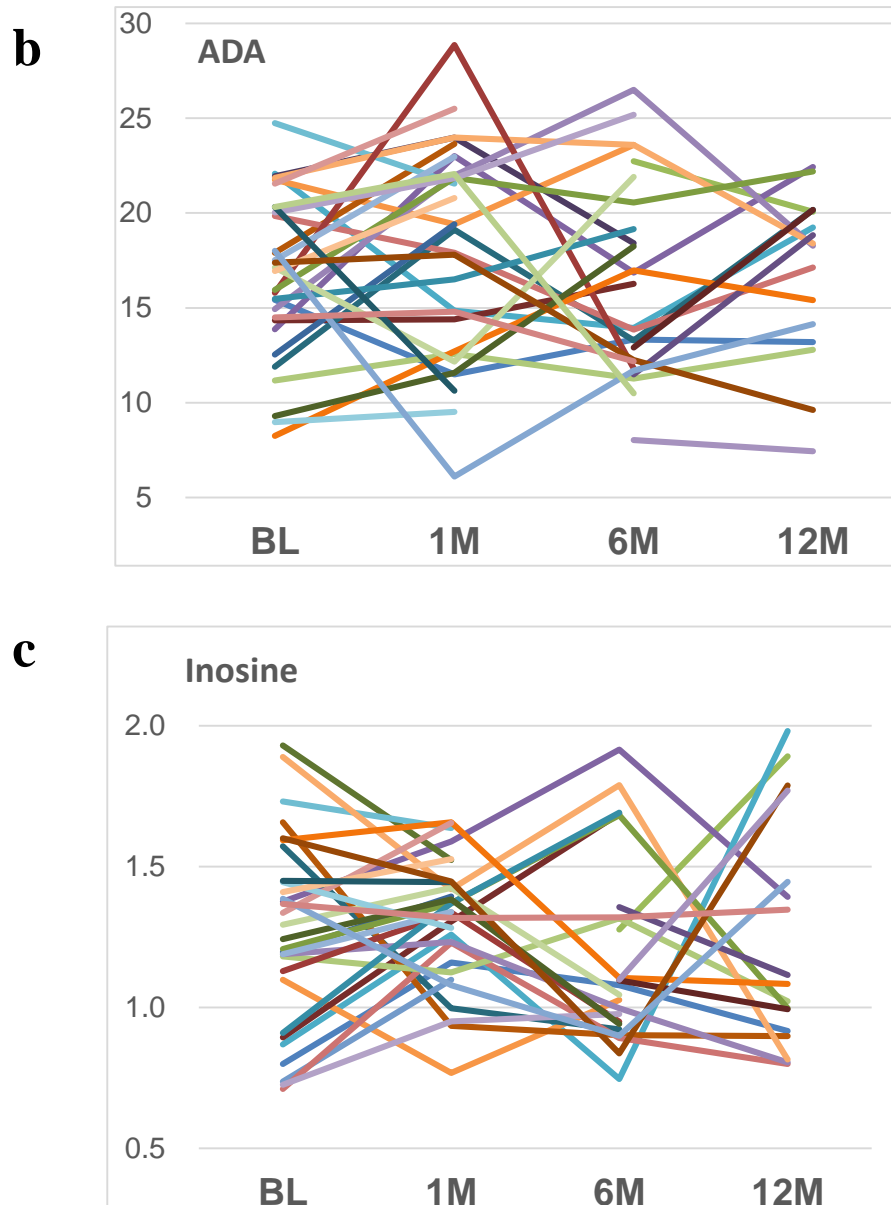


Figure 16. Trajectory chart of (a) adenosine, (b) ADA activity and (c) inosine levels in the longitudinal study.

Each line represents for the trajectory of each subject from baseline to 12 months after treatment. Some lines are not contiguous due to missing values of those analyte levels in follow-up groups. Detailed demographic information of this study group can be found in table 4.

Abbreviations: ADA = adenosine deaminase; HC = healthy control; BL = baseline; 1M = 1 month after baseline; 6M = 6 months after baseline; 12M = 12 months after baseline. Units: adenosine and inosine levels are in μM , ADA levels are in I/U.

3.3.6 Correlations between adenosine and ADA in BL and post-treatment groups

We then compared correlations between adenosine and ADA among BL and post-treatment groups. We didn't find significant correlations between adenosine and ADA in BL or any post-treatment groups. However, after long-term treatment (12M), the correlation became more significant when compared with short-term treatment (Figure 16).

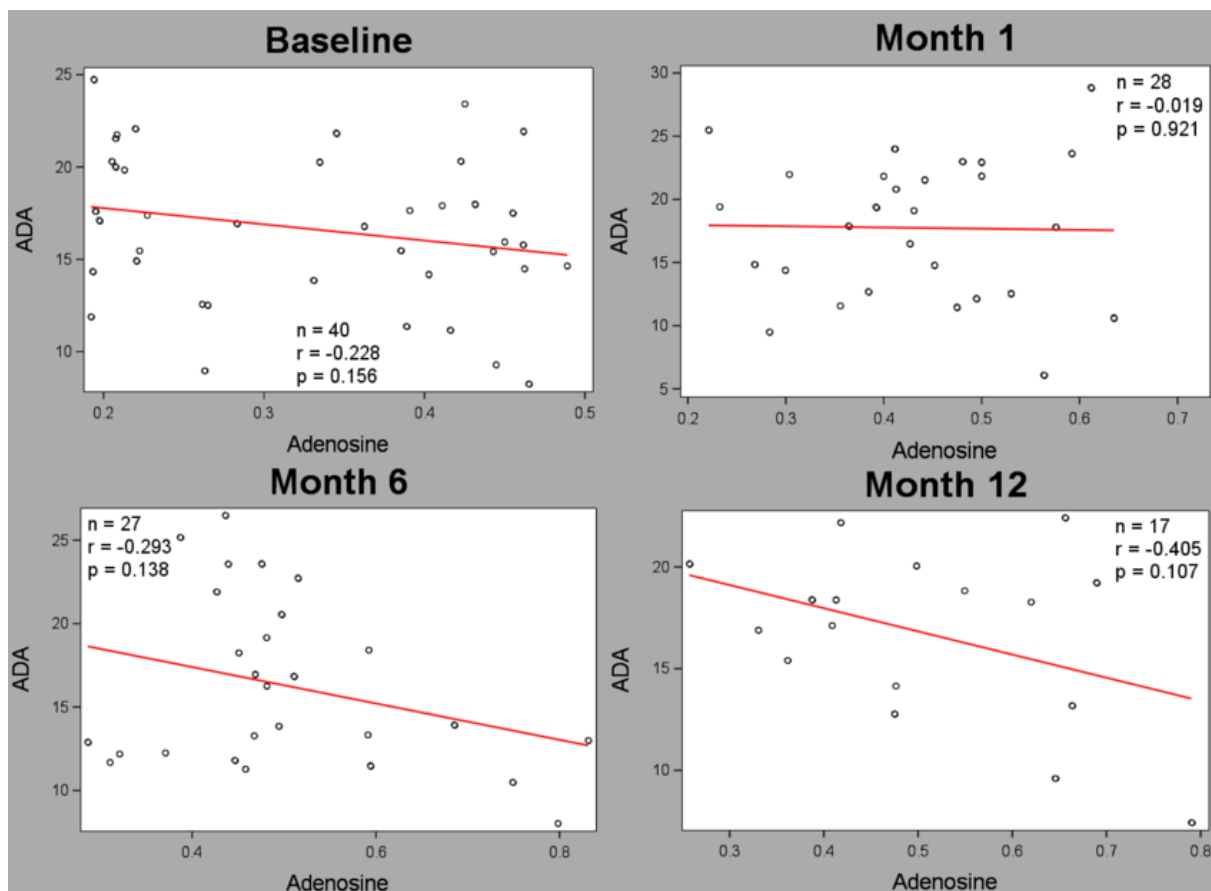


Figure 17. Correlations between adenosine and ADA in baseline and post-treatment groups.

Abbreviations: ADA = adenosine deaminase. Units: adenosine and inosine levels are in μM , ADA levels are in I/U.

3.4 DISCUSSION

Although accumulating evidence have suggested that ARs (especially A_{2A}R) agonists could be the potential new generation of antipsychotics (Rimondini et al. 1997, Dixon et al. 1999, Bridges et al. 1987), D₂R blocker remains the front-line antipsychotics in treating SZ patients at present time. Both first- and second-generation antipsychotics showed a high binding affinity of the D₂R in striatum. As mentioned in the instruction section, activation of ARs would have inhibitory effect on D₂R which is similar to the D₂R blocker's effect. In fact, when previous study looked into behavioral assays to predict antipsychotic activity, AR agonists block behaviors in a similar manner to dopamine receptor antagonists in animal model (Kafka and Corbett. 1996). Vice versa, clinical study showed that in SZ postmortem striatum, AR density was positively correlated with the dose of antipsychotics when those SZ patients were treated with chlorpromazine (D₂R blocker) equivalents, implying an up-regulation of adenosine signaling by D₂R blockers (Deckert et al. 2003). Based on robust pharmacological data foundations that supporting antipsychotics' effect on adenosine signaling via D₂R-AR interactions (Ferré 1997, Ferré et al. 1994, Akhondzadeh et al. 2000), we hypothesized that antipsychotics can affect and restore analytes' levels that related to adenosine signaling in SZ patients. Results in this chapter partially supported our hypothesis.

Findings in 3.3.1 showed that antipsychotics can affect adenosine signaling in CNS. No significant difference was found between on-treatment SZ group and HC group in terms of inosine and ADA activity levels, which indicated that antipsychotics can normalize inosine and

ADA activity levels. These findings support the hypothesis that antipsychotics can at least partially restore back the adenosine signaling in the CNS. Combined with findings in chapter 2, CSF inosine and ADA activity levels may serve as potential therapeutic indicators. While treatment can also significantly restore adenosine levels in on-treatment SZ group, significantly lower adenosine levels in off-treatment group were still found when compared to those in HC group. This observation suggested that those alterations of analyte levels along with the adenosine signaling deficits which were implied by observations in the SZ patients might be both trait and state features of SZ. Furthermore, such differences might reflect the limitation of current therapy which was based on the classical dopamine theory, and further contribute to negative & cognitive symptoms that are poorly responsive to current antipsychotics. In other words, D₂R blocker cannot fully restore back the adenosine signaling in the CNS, and that might explain D₂R blocker's weakness in treating negative & cognitive symptoms.

Significantly higher adenosine levels in on-treatment SZ patient plasma indicated that treatment may also affect peripheral tissues. Similar to our findings in CSF, we observed significantly lower plasma adenosine levels in on-treatment SZ group than those in HC group, which also indicated that treatment cannot fully restore adenosine levels in peripheral tissues. However, plasma ADA activity and inosine levels were not significantly changed by treatment as situations in CSF. It looks like treatment has little impact in peripheral tissues, but if took findings in second chapter into consideration, findings with regard to analytes' levels that were observed that were not significantly changed by antipsychotics may be reasonable. When comparing ADA activity and inosine levels in plasma between FEANS group and HC group

in 2.3.5, we also did not find significant differences. Therefore, we speculate that adenosine signaling in SZ is less altered in peripheral tissues than that in the CNS, and antipsychotics thus have less normalizing effect in peripheral tissues. More studies are needed to test differences of between peripheral tissues and CNS in terms of inosine & ADA activity levels.

As for the correlation between adenosine and ADA, results in 3.3.3 showed that correlations between adenosine and ADA could be partially restored by treatment, but based on our findings, this normalizing effect was only limited to peripheral tissues. The unchanged correlation between adenosine and ADA in the CSF after the treatment suggested that current treatment cannot normalize the disrupted connections within adenosine signaling in the CNS.

After validation of the treatment effect on adenosine signaling, we then tested if short-term treatment effects differ from the long-term ones in longitudinal study. Due to the differences of sample sizes and covariates among BL and post-treatment groups, we used mixed linear model in this study. Findings in Table 7 showed that age and race were significant covariates in fixed effect test, although relationships between adenosine and ADA was still significant while including age and race into the model. Based on published literature, no evidence has been suggesting race-related difference on adenosine signaling in human. With regard to age, one *in vitro* study suggested that responsiveness of the beta-adrenergic system in human vascular smooth muscle (Ford et al. 1992) was associated with age. Another animal study reported that the reduction of AR expression in rat heart (Jenner et al. 2004) might also be age-associated. But overall we did not find robust data directly support that age can significantly affect adenosine signaling.

With regard to analytes' levels among BL and post-treatment groups in first-episode SZ patients, first, we found similar changing pattern of analytes' levels by antipsychotics in this study (risperidone, second generation antipsychotics) in comparison of on-/off-treatment chronic SZ group (haloperidol, first generation antipsychotic). It validated previous pharmacological findings that antipsychotics affect adenosine signaling through AR-D₂R interactions instead of AR-serotonin receptor interactions. As for comparisons made between post-treatment analytes' levels and baseline analytes' levels, findings here divergently validated the result in 3.3.2, suggesting that antipsychotics can significantly elevate plasma adenosine levels while having little impact on ADA activity and inosine levels. Within-subjects trajectory charts also supported this finding by showing significantly higher levels of adenosine in over 90% of subjects after the treatment in comparison with those in BL. Furthermore, no significant difference was observed between short- and long-term treatment effects with regard to adenosine, inosine and ADA activity levels. It indicated that when patients are on stable doses, adenosine, ADA activity and inosine levels are also in stable conditions. Trajectory analysis also supported this finding by showing no significant change over time among all post-treatment groups. As for post-treatment analytes' levels vs HC levels, however, it is important to note that adenosine levels in post-treatment groups was no longer significantly different with those in HC group. This finding is not consistent with findings in 3.2.1 and 3.2.2 when we were comparing on-treatment chronic SZ group and HC group. It may indicate that antipsychotics have the potential to fully restore adenosine levels at the early stage of the disease, but such capacity is lost as disease progresses to chronic stage.

At last, we checked connections among adenosine, inosine and ADA in BL and post-treatment groups. The hypothesis that ADA is the mediator of the conversion from adenosine to inosine was rejected by fixed effect tests. Also, inosine levels cannot be predicted by ADA or adenosine or ADA & adenosine levels in any of SZ groups. No significant correlation between adenosine and ADA was observed in BL any of the post-treatment groups. These findings suggested that adenosine signaling might not be the only part that was disrupted by SZ in purine pathway. Inosine and following enzymatic reactions may also get altered in SZ so that adenosine and ADA activity levels cannot predict inosine levels. Furthermore, these disrupted connections cannot be significantly improved by antipsychotics in the short-term. However, it tends to show a more significant correlation between adenosine and ADA in long-term treatment (12M) group, which is similar with results in figure 14 (chronic on-treatment SZ group). These correlations were in inversed pattern which were same as the one in HC group. Mixed model analysis also showed that ADA activity levels could be predicted by adenosine levels during the treatment over time. These findings implied that treatment can gradually improve part of the connection between adenosine and ADA over time.

3.5 CONCLUSION

1. Results in this chapter indicated that antipsychotics can affect adenosine signaling in SZ patients. Results supported the hypothesis that treatment can at least partially restore adenosine, inosine and ADA activity levels in the CSF. Interestingly, plasma adenosine levels in first-

episode SZ group is not significantly different with levels in HC group after the treatment, while in chronic SZ on-treatment group it is still significantly lower than those in HC group. More studies are needed to check if antipsychotics could better restore adenosine levels at the early stage of the disease than that in the chronic stage.

2. We found significant changes of ADA activity and inosine levels in on-treatment SZ group when compared to off-treatment group in the CSF. However, we did not see significant changes of these analytes' levels in the plasma either between on- and off-treatment groups or between baseline and post-treatment groups. It suggested that antipsychotics affect adenosine signaling more in the CNS than in peripheral tissues.

3. Findings indicated that antipsychotics can normalize inosine and ADA activity levels which were altered by SZ (results shown in 2.3.2) in the CNS. Therefore, CSF inosine and ADA activity levels may serve as potential therapeutic indicators.

4. Correlation analysis implied that treatment can gradually improve part of the connection between adenosine and ADA over time. Nevertheless, we didn't see any of the post-treatment group showed significant correlation between adenosine and ADA. Also, adenosine levels in the CSF were observed that have significantly increased but still cannot be fully restored by the antipsychotics. These observations suggested that those changes of analyte levels in the SZ might be both trait and state features. Also, these results implied that current antipsychotics cannot fully normalize the disrupted adenosine signaling, which may contribute to current antipsychotic's weakness in treating negative & cognitive symptoms among SZ patients.

Overall, findings in this chapter supported the central hypothesis that antipsychotics can affect and partially restore the adenosine signaling in SZ patients. Combined with findings in Chapter 2, adenosine signaling deficits which were suggested by data here are more likely to be trait and state features of SZ simultaneously. Also, results suggested that adenosine signaling modulator might be the silver lining in the endeavors of new antipsychotics R&D.

4.0 HOMEOSTATIC IMBALANCE OF PURINE PATHWAY IN SCHIZOPHRENIA

4.1 BACKGROUND

In human cells, purines are synthesized *de novo* from amino acids, formate and carbon dioxide as nitrogen and carbon donors. Adenosine signaling also serves as a signaling mechanism that is associated with the purine pathway. After being synthesized, adenosine is then metabolized to inosine, hypoxanthine, xanthine and ends in uric acid. In human, uric acid is the final product of purine catabolism (Linden and Rosin. 2006) and serves as a potent antioxidant and accounts for up to 65% of the total antioxidant capacity in the body (Wayner et al. 1987, Miller et al. 1993). We previously reported a significant decrease of plasma uric acid levels in SZ patients (Yao et al. 1998), which linked the potential purine pathway homeostatic alterations to the oxidative stress.

It has long been proposed that at least in part, the neuropathological changes in SZ may result from oxidative stress mechanisms (Yao et al. 2001, Do et al. 2009, Fendri et al. 2006, Boskovic et al. 2011 and Flatow et al. 2013) while the precise components of such neuropathological alterations remain unclear. Under normal physiological conditions, free radical-mediated damage is opposed by the antioxidant defense system (AODS), which consisted a series of enzymatic and non-enzymatic components. These components act cooperatively at different sites in the free radical pathways. Under disease state, the AODS is compromised (Virit et al. 2009, Yao et al. 2006, Ranjekar et al. 2003) due to increased reactive

oxygen species (ROS) levels (Brieger et al. 2012, Koga et al. 2016). Consequently, unchecked ROS then triggers series of pathological processes such as DNA damage, lipid peroxidation and metabolic defects. In this dissertation, the metabolic defect we are interested in is the purine metabolism.

In chapter 2 and chapter 3, we have observed adenosine signaling deficits in SZ patients which could be affected by antipsychotic therapy. In this chapter, we would like to explore the possible cause of such deficits. Adenosine theory states that it is the dysfunction of purinergic system that leads to the adenosine signaling deficits (Lara et al. 2006). Based on previous findings, we hypothesize that in SZ, an increased oxidative stress would cause the breakdown of AODS. As an important component of AODS, uric acid levels are consequently reduced. The reduction of the end product of purine pathway (uric acid) would lead to the metabolic shift of purine metabolism and eventually result in adenosine signaling deficits.

To support this hypothesis, we tested three sub-aims in this chapter. Results & discussion are based on the flow of following sub-aims. This chapter also serves as a part of the aim 3 in dissertation.

Aim 3.1.a. Test if homeostatic imbalance of purine pathway presents in SZ patients. We measured six major metabolites (guanosine, guanine, xanthine, xanthosine, hypoxanthine and uric acid) in the purine pathway other than adenosine, ADA and inosine. Comparisons of analytes' levels were made between FEANS group and HC group. Then we compared ratios of product to precursor and detected the possible shift of the pathway in FEANS group in comparison to HC group.

Aim 3.1.b. Test if uric acid's level reduction leads to adenosine signaling deficits in SZ. Here we checked the correlations between adenosine signaling and the end product (uric acid) of purine pathway in both FEANS and HC groups.

Aim 3.1.c. Test if oxidative stress plays a role in causing uric acid's level reduction. Here we measured levels of nitric oxide (NO) and malondialdehyde (MDA) in order to reflect oxidative stress levels. NO generates from L-arginine by nitric oxide synthase. After reacting with superoxide radicals, it then form peroxynitrite and contribute to oxidative stress level. NO is therefore been regarded as the marker of oxidative stress (Pierini and Bryan, 2015). MDA is the byproduct of lipid peroxidation and is generally used as the marker of lipid peroxidation (Gawel et al. 2004, Del Rio et al. 2005). Besides uric acid levels, we also measured total antioxidant status (TAS) to reflect the total antioxidant capacity of the plasma in SZ patients and controls. First, we compared levels of these markers between FEANS group and HC group. Second, we tested the correlations between these markers and uric acid.

4.2 EXPERIMENTAL PROCEDURES

4.2.1 Clinical design

Forty-two SZ patients were recruited from clinical services of the VA Pittsburgh Healthcare System (VAPHS). All procedures were approved by the VAPHS Highland Drive Institutional Review Board. After providing informed consent for their participation, patients were recruited

after having had at least two years of treatment on antipsychotics. Diagnostic assessments and clinical symptom ratings were performed by experienced research clinicians. Blood samples were collected and clinical symptoms were evaluated prior to initiation of clinicians' choice of antipsychotic agents. Blood cotinine levels were measured and those patients who had levels over 10ng/mL were considered to be smokers. In addition, 53 age-, race-, BMI-, smoking habit- and gender-matched HC subjects were also recruited for comparison. The demographic table of subjects is shown below (Table 9).

Table 9. Demographic table of recruited subjects

	HC	FEANS
Numbers	53	42
Gender ^a	32/20	26/14
Age	26.0 ± 7.4	22.8 ± 8.0
BMI	25.1 ± 4.4	23.4 ± 5.2
Race ^b	34/11/3/4	22/16/2/2
Smoking ^c	32/21	26/16

^a Male/Female. ^b Caucasian / African American / Pacific Asian / others. ^c Smoker/non-smoker

Abbreviations: HC, healthy controls; FEANS, first episode antipsychotic naïve schizophrenia patients; BMI, body mass index. Note: age and BMI data are expressed in mean ± standard deviation; in HC group, one subject is missing gender and race information. Three subjects in FEANS group and one in DC group are missing BMI data due to missing height/weight values.

4.2.2 High-pressure liquid chromatography assay

Guanoine, guanine, xanthine, hypoxanthine, xanthosine and uric acid were measured through high-pressure liquid chromatography coupled with electrochemical coulometric array system (HPLC-CMEAS). After centrifugation at 3000 rpm for 20min, 120 μ L plasma samples including the internal standard (3, 4-Dihydroxybenzylamine) at 500ng/mL were injected into the HPLC system. Each sample was tested in duplicates and QCs were used to check the differences among different batches. Detailed methodology had been described earlier (Kristal et al. 2007, Shi et al. 2002). Briefly, the liquid chromatographic method employs an A mobile phase (10.3 g/L sodium pentane sulfonate, 5 ml/L glacial acetic acid) and a B mobile phase (methanol/acetonitrile/isopropanol 8/1/1, 8 g/L lithium acetate, 20 ml/L glacial acetic acid). A gradient is run from 100% A to 100% B over 65 min. The electrochemical array of 12 series coulometric detectors is set from 0 to 900 mv in equal 60 mv increments from detector 1 to 12. In this mode, the compound passing through a coulometric electrode is oxidized by 100% of the thermodynamically possible amount. Results were in a characteristic signature for a compound expressed as a ratio on sequential electrodes. All chromatograms in this study were background-corrected to eliminate the base line drift inherent in gradient profiles. All responses matching the retention and electrochemical signature of compounds in the reference standard were exported in concentration units of ng/ml. Linear standard curve range of this assay is from 2.5 ng/mL to 100 μ g/mL. The Lower Limit of Quantification is 1.0 ng/mL. Intra assay percentage of CV is below 1.5% and inter assay percentage of CV is below 2.0%.

4.2.3 Enzyme-linked immunosorbent assay

MDA was measured through ELISA kit from Abcam (ab118970). Linear standard curve range is from 1.33 μM to 20.67 μM . The Lower Limit of Quantification is 0.05 μM . Intra assay percentage of CV is below 3.0% and inter assay percentage of CV is below 4.5%. TAS was measured through ELISA kit from Cayman (709001). Linear standard curve range is from 10 μM to 100 mM. The Lower Limit of Quantification is 1 μM . Intra assay percentage of CV is below 5.0% and inter assay percentage of CV is below 8.0%. NO was measured through ELISA kit from Abcam (ab65328). Linear standard curve range is from 0.45 μM to 90 μM . The Lower Limit of Quantification is 0.33 μM . Intra assay percentage of CV is below 5.0% and inter assay percentage of CV is below 6.0%. Reading was obtained from PerkinElmer micro plate reader (Wallac 1420). MDA data was read at 530 nm, TAS data was read at 410 nm, and NO data was read at 540 nm. Each sample was tested in duplicates and QCs were used to check the differences between plates.

4.2.4 Data analysis

Comparisons of analytes' levels were made between FEANS and HC groups via sample t test. Correlations of analytes were also analyzed in both FEANS and HC groups via linear regressions. All raw data and results were checked for distribution via quantile-quantile plots and main findings were summarized thereafter. All tests were two-tailed.

4.3 RESULTS

4.3.1 Comparisons of major metabolites' levels in purine pathway between FEANS group and HC group

As shown in Table 10, comparisons of purine pathway metabolites were made between FEANS group and HC group. Significant decrease of adenosine, hypoxanthine, guanine and uric acid levels were observed in FEANS group in comparison of HC group. No significant difference was found in inosine, xanthine, xanthosine and guanosine levels between FEANS group and HC group.

Table 10. Comparisons of purine metabolites' levels between FEANS and HC groups

Metabolite	HC		FEANS		p value
	Mean	STDEV	Mean	STDEV	
Adenosine	130	24	88	18	0.0003
Inosine	308	55	341	62	0.052
Hypoxanthine	1785	310	1071	211	<0.0001
Xanthine	151	39	180	44	0.011
Xanthosine	19	4.7	21	5.0	0.566
Guanine	7.2	1.7	4.6	1.4	<0.0001
Guanosine	29	7.4	27	7.0	0.856
Uric acid	50.1	9.3	35.1	7.9	<0.0001

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve schizophrenia patients; STDEV = standard deviation. Means and standard deviations of uric acid are in $\mu\text{g/mL}$. All other levels of metabolite means and standard deviations are in ng/mL . Critical p value after Bonferroni correction: 0.0063.

4.3.2 Ratios of product to precursor in purine pathway between FEANS group and HC group

As shown in table 11, ratios of product to precursor were calculated and comparison of these ratios were made between FEANS group and HC group. In most cases, product/precursor ratios are higher in FEANS group than those in HC group except UA/Xant, Xant/Gr and G/Gr, showing a general trend of pathway shift from precursors to products in SZ.

Table 11. Comparisons of product to precursor ratios between FEANS and HC groups

Product/Precursor	HC		FEANS		p value
	Mean	STDEV	Mean	STDEV	
Ino/Ade	2.7	1.3	4.3	1.8	<0.0001
Xan/Hx	0.09	0.04	0.18	0.06	<0.0001
Xan/Ade	1.4	0.8	2.3	1.1	<0.0001
G/Gr	0.26	0.13	0.17	0.09	0.003
Xan/G	30	21	64	43	<0.0001
UA/G	10.5	9.7	13.0	10.5	0.022
UA/Xant	2878	1176	1823	762	0.001
Xant/Gr	0.77	0.47	1.07	0.81	0.038
Xan/Xant	8.7	4.1	9.5	4.9	0.29

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve schizophrenia patients; STDEV = standard deviation; Ade = adenosine; Ino = inosine; Gr = guanosine; G = guanine; Xan = xanthine; UA = uric acid; Xant = xanthosine; Hx = hypoxanthine. Critical p value after Bonferroni correction: 0.0063.

4.3.3 Homeostatic imbalance of the purine pathway in SZ

We then graphed the metabolic map in order to reflect purine metabolic alterations in SZ. As shown in figure 17, a metabolic shift from adenosine and guanosine to xanthine was observed.

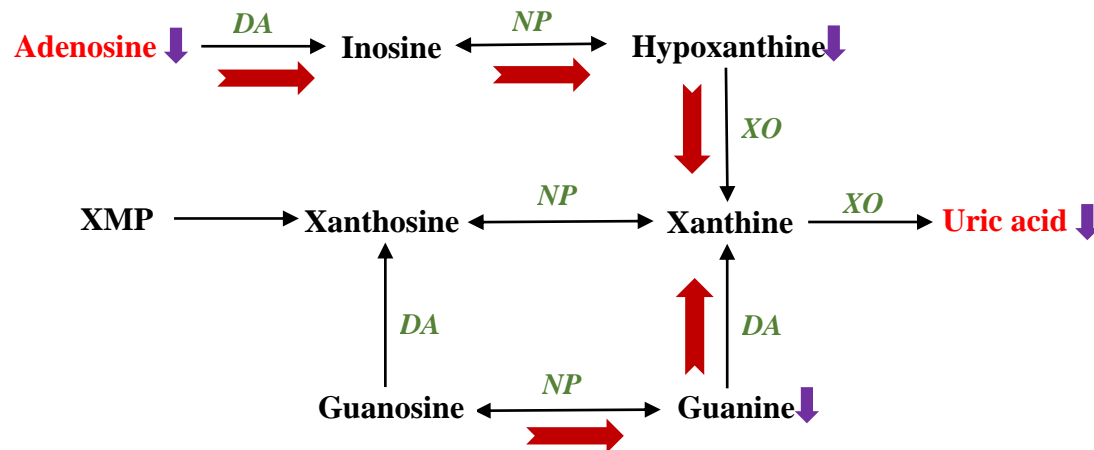


Figure 18. Altered purine metabolism in FEANS group.

Purple arrows show the changes of metabolites' levels and red arrows show the metabolic shift directions. Abbreviations: NP = nucleoside phosphorylase; DA = deaminase; XO = xanthine oxidase; XMP = xanthosine monophosphate.

4.3.4 Correlations between levels of analytes in adenosine signaling and levels of uric acid in HC and FEANS groups

To test if adenosine signaling correlated with the end product of purine pathway, we checked correlations between adenosine signaling analytes and uric acid in HC and FEANS groups. Significant trend of correlation was found between uric acid and adenosine in HC groups while significant correlation was found between uric acid and adenosine in FEANS groups.

Significant inverse correlations was found between uric acid and ADA in both HC and FEANS groups (Figure 18).

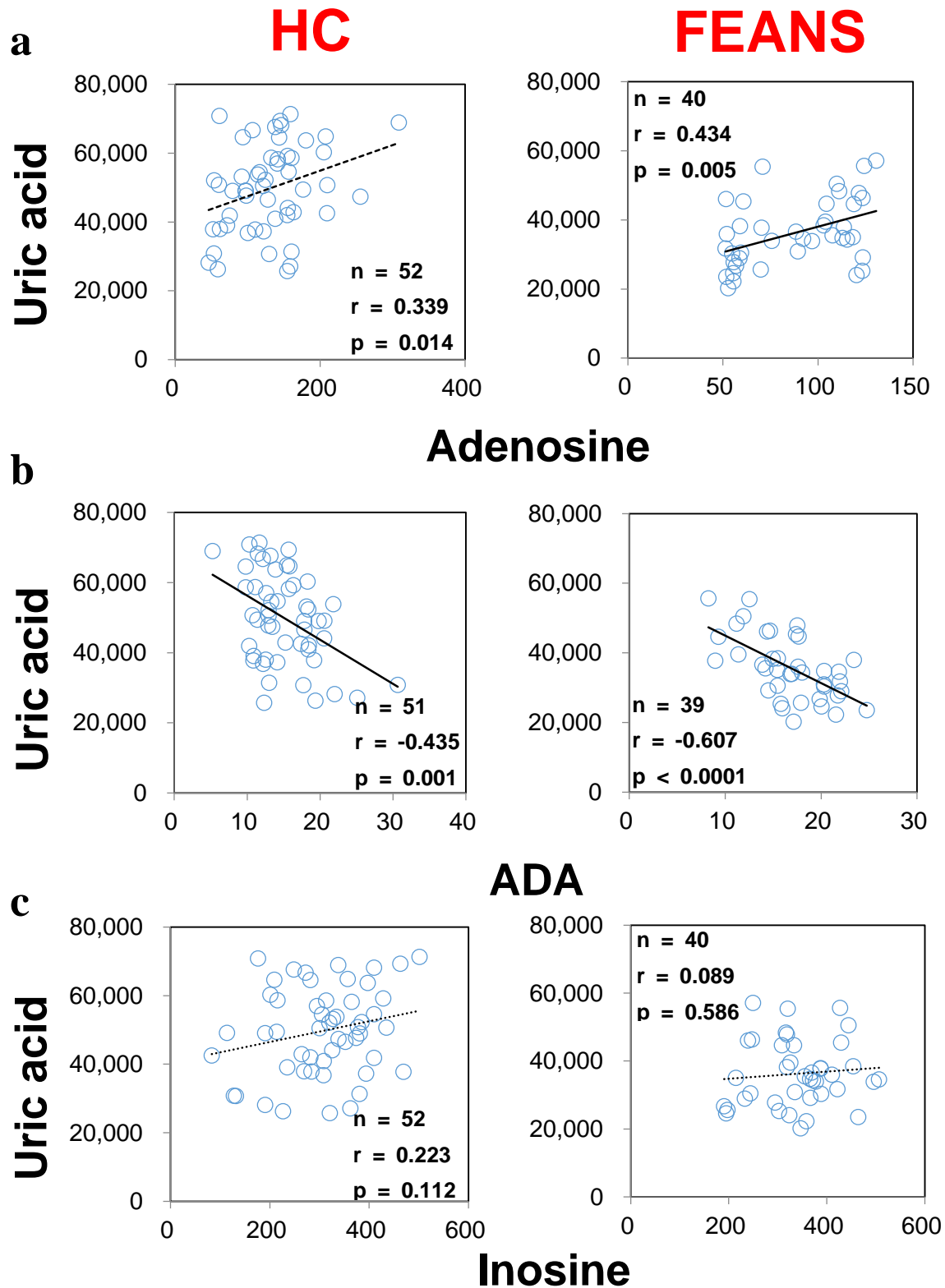


Figure 19. Correlations between uric acid and (a) adenosine, (b) ADA and (c) inosine in HC and FEANS groups.

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve SZ patients; ADA = adenosine deaminase. Critical p value after Bonferroni correction: 0.0083 (significant), 0.0167 (trend). Units: ADA is in I/U; Adenosine, uric acid and inosine are in ng/mL.

4.3.5 Comparisons of levels of oxidative stress markers and total antioxidant status between HC and FEANS groups

Here we tested if levels of the oxidative stress marker (NO) and the lipid peroxidation marker (MDA) were different in FEANS group in comparison with those in HC group. We also compared TAS levels between these two groups to test if AODS were altered in SZ. As shown in Figure 19, significantly lower levels of TAS was found in FEANS group in comparison with those in HC group. Significantly higher levels of NO and MDA were found in FEANS group in comparison with those in HC group.

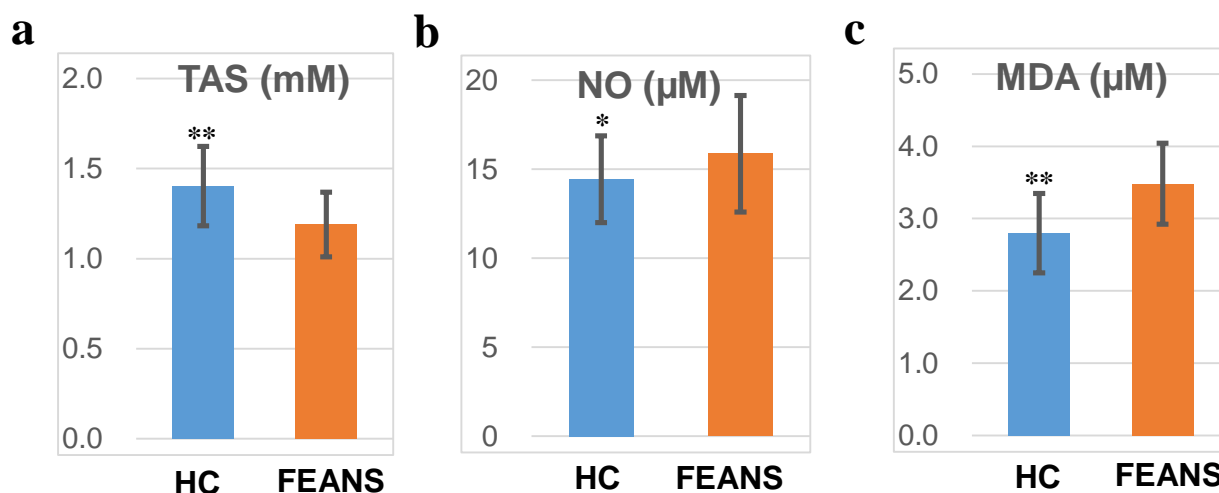
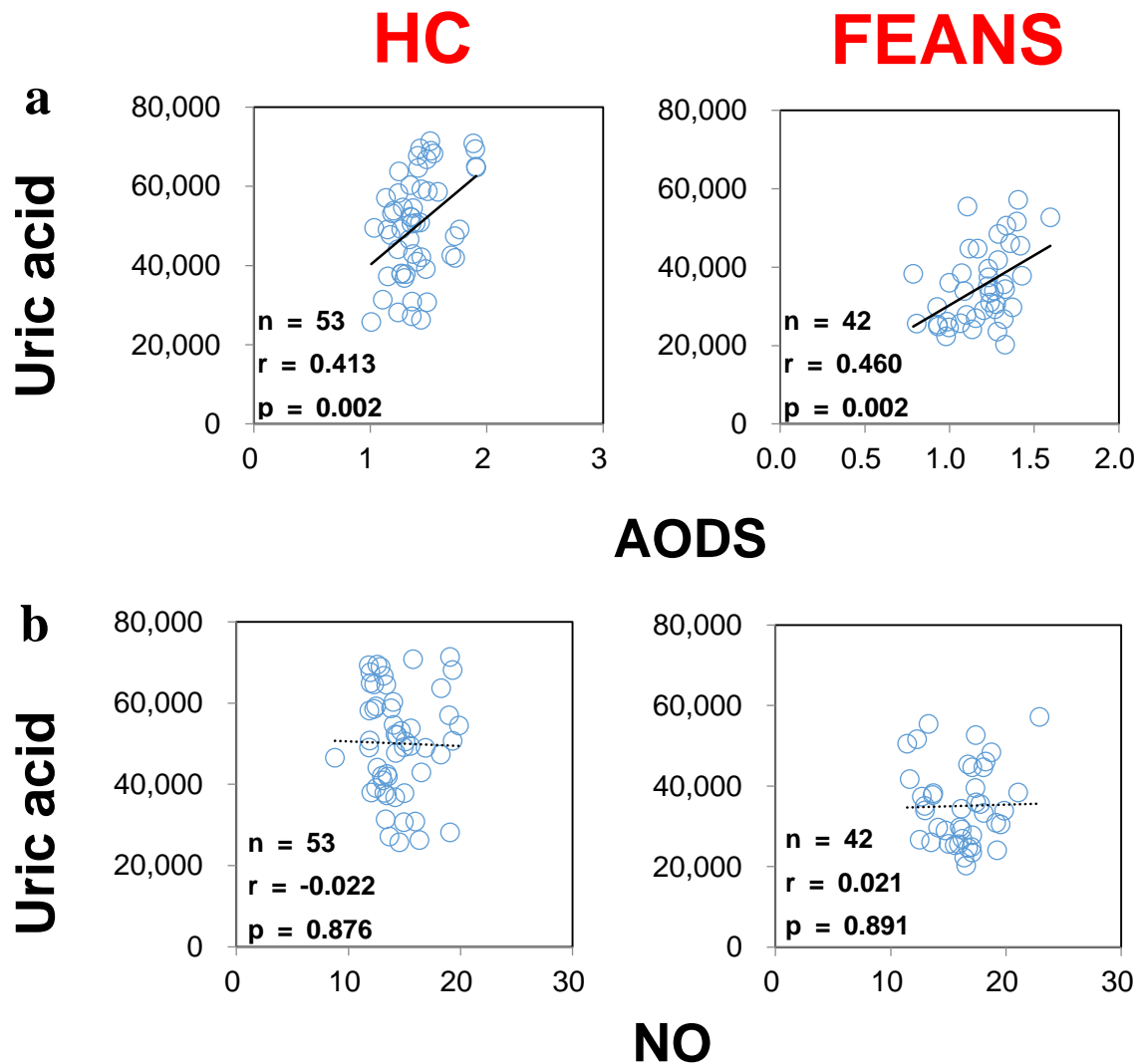


Figure 20. Comparisons of (a) TAS, (b) NO and (c) MDA levels between HC and FEANS groups.

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve SZ patients. *, p < 0.01; **, p < 0.001.

4.3.6 Correlations between oxidative stress markers and uric acid in HC and FEANS groups

At last, we tested whether these indices that mentioned above are correlated with uric acid in HC and FEANS groups (Figure 20). Significant correlations were observed between uric acid and AODS in both HC and FEANS groups. Also, significant inverse correlations were found between uric acid and MDA in both HC and FEANS groups. On the other hand, we didn't find any significant correlations between uric acid and NO in either HC or FEANS group.



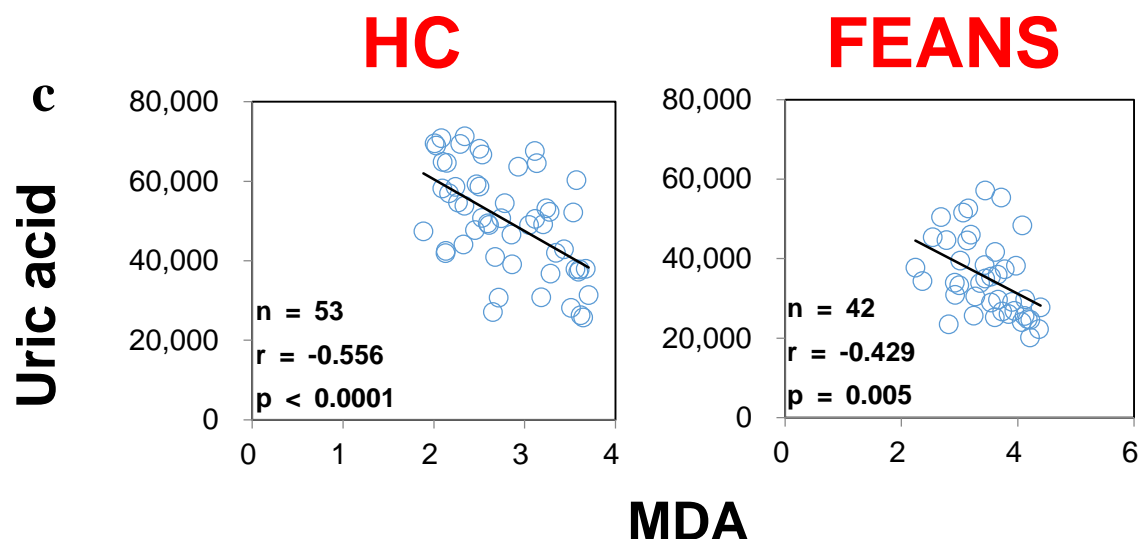


Figure 21. Correlations between uric acid and (a) AODS, (b) NO and (c) MDA in HC and FEANS groups.

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve SZ patients; ADA = adenosine deaminase. Critical p value after Bonferroni correction: 0.0083. Units: uric acid is in ng/mL; AODS is in mM; NO and MDA are in μ M.

4.4 DISCUSSION

In this chapter, three specific questions were tested and answered here. First of all, we tested if the alterations of purinergic signaling present in SZ. Second, we tested if such alterations can be linked to adenosine signaling deficits. Third, we tested if oxidative stress plays a role in causing purinergic signaling alterations.

Among eight purine metabolites that were analyzed by HPLC, four of them (adenosine, hypoxanthine, guanine and uric acid) were observed that levels were significantly lower in FEANS group than those in HC group. The other four metabolites' levels in FEANS group

were not significantly different with those in HC group. No significantly increased levels of any metabolite were observed in FEANS group when comparing to HC group. These findings implied that there might be a hypofunction of purinergic signaling in the SZ, and adenosine deficits belongs to a part of it. This probably explained that we could not predict inosine levels solely by adenosine and ADA in previous chapters. However, by comparing ratios of product to precursor, we were able to estimate the possible shift of the metabolism in SZ. Specifically, we observed that the pathway shifts from adenosine and guanosine to the xanthine in FEANS group when comparing with the pathway in HC group. Xanthine could be metabolized by nucleoside phosphorylase to xanthosine (reversible) or by xanthine oxidase to uric acid. Since in FEANS group we have found a significant decrease of uric acid levels while xanthosine levels were not been significantly changed, we speculated that it is the decreased levels of uric acid that drives such metabolic shift.

To further validate this assumption, we tested if uric acid levels can correlate with the adenosine signaling. Significant or significant trend of correlations between uric acid and adenosine implied that a reduced levels of uric acid associated with reduced levels of adenosine in purine metabolism. Significantly inversed correlation between uric acid and ADA suggested that a reduced levels of uric acid may lead to a higher activity levels of ADA, so that the metabolism could be shifted in favor of the uric acid production.

We next looked for the possible reason that leads to the decreased levels of uric acid in SZ patients. Since numerous studies linked oxidative stress in SZ pathology (Yao et al. 2001, Do et al. 2009, Fendri et al. 2006, Boskovic et al. 2011 and Flatow et al. 2013) and uric acid

serves as an important component of AODS, we then tested the relationship between oxidative stress and uric acid. We chose MDA, NO and TAS as oxidative stress markers in this chapter based on previously established principles (Ho et al. 2013, Ghiselli et al. 2001). In consistent with previous findings (Das et al. 1995, Yao et al. 2004, Yao et al. 1998, Li et al. 2011, Zhang et al. 2007, Kuloglu et al. 2007), significantly higher levels of MDA & NO and significantly lower levels of TAS was found in FEANS group in comparison to HC group. With regard to correlations, significant correlations were observed between uric acid and AODS. Also, significant inverse correlations were found between uric acid and MDA. These findings suggested that uric acid may play an important role in the lipid peroxidation process and AODS. On the other hand, we didn't find any significant correlations between uric acid and NO in either HC or FEANS group. This finding suggested that uric acid might not protect cells from ROS through the arginine-nitric oxide pathway. Future studies are required to reveal the underlying mechanism of uric acid's protection against oxidative stress in the body.

4.5 CONCLUSION

1. Based on findings in this chapter, it is suggested that a hypofunction and homeostatic imbalance of purine pathway are associated with the SZ pathology. Interestingly, results suggested that adenosine, ADA and inosine are not the only parts of the purine pathway that has been altered by SZ. Since purine pathway has not been fully studied in the SZ pathology,

more data and evidence are needed to illustrate such metabolic defects and the potential clinical meanings behind it.

2. Data suggested that purine pathway shifts from adenosine and guanosine to the xanthine. Results suggested that such shift may result from the compensatory response to the reduced uric acid levels in SZ patients. Significant correlations were noted between uric acid and analytes in the adenosine signaling, implied that adenosine signaling deficits may result from the metabolic shift of pathway which was initially caused by the decreased level of uric acid.
3. Results suggested that oxidative stress may play an important role in causing the decreased level of uric acid. Increased levels' oxidative stress markers and decreased TAS levels were observed in SZ, which supported the role of oxidative stress in SZ pathology. Significant correlations between uric acid and these markers suggested that oxidative stress may compromise AODS and reduce uric acid levels, and eventually triggers purine pathway metabolic defects.

Based on findings in this chapter, we speculate that due to genetic or environmental factors, an increased oxidative stress level presents in SZ patients. The accumulation of oxidative stress in the body compromises the AODS and reduces uric acid levels in the body. The reduction of uric acid would lead to the compensatory response of the purine pathway and the metabolism would shift towards the direction in favor of uric acid production. As a consequence of metabolic shifts, adenosine signaling on the other end is then compromised and eventually developed into deficits.

5.0 ASSOCIATIONS OF ADENOSINE SIGNALING DEFICITS AND MEMBRANE LIPID DEFECTS IN SCHIZOPHRENIA

5.1 BACKGROUND

Lipid theory of SZ pathology states that an increased lipolysis of membrane phospholipids in the body system results in SZ symptoms. Specifically, previous findings (Gattaz et al. 1990, Pettegrew et al. 1991, Horrobin et al. 1991 and Nojonen et al. 1993) indicated that in both CNS and peripheral tissues, an increased lipolysis of membrane phospholipids presents in SZ patients due to an increased PLA₂ activity. Interestingly, adenosine's role in mediating lipid metabolism especially on lipolysis via adenosine receptors has been highlighted by previous studies (Schwabe et al. 1974, Johansson et al. 2008). Details of the adenosine's regulation on lipolysis are introduced in section 1.4. Under such context, we would like to test if adenosine signaling correlates to SZ pathology through the regulation of membrane lipid lipolysis.

This chapter also serves as a part of the aim 3 in dissertation. We tested two sub-aims in this chapter. Results & discussion are based on the flow of following sub-aims.

Aim 3.2.a. Test metabolic defects during the lipolysis of membrane phospholipids in FEANS group versus HC group. We are interested in studying membrane phospholipid lipolysis and resulting free fatty acids (FFAs) in SZ. As shown in figure 21, after the lipolysis from membrane phospholipids, FFAs serve as an active circulating pool in the body. They involve in many important biochemical reactions including regeneration of membrane

phospholipids, oxidation and production of prostaglandins (Zhou et al. 2017, Zhou et al. 2018).

To test the lipid theory of SZ, we compared the total level of esterified fatty acids (FAs) in RBC samples and FFAs levels & profiles in plasma samples between FEANS and control groups. As an essential lipid constituent of cell membranes, membrane cholesterol levels in RBC samples among FEANS and control groups were also measured and compared between FEANS and HC group. In DC group, we did not measure esterified FAs and cholesterol levels.

Aim 3.2.b. Test if adenosine correlates to lipid metabolic defects. We tested the correlations between adenosine and analytes (FAs, FFAs and cholesterol) that mentioned above.

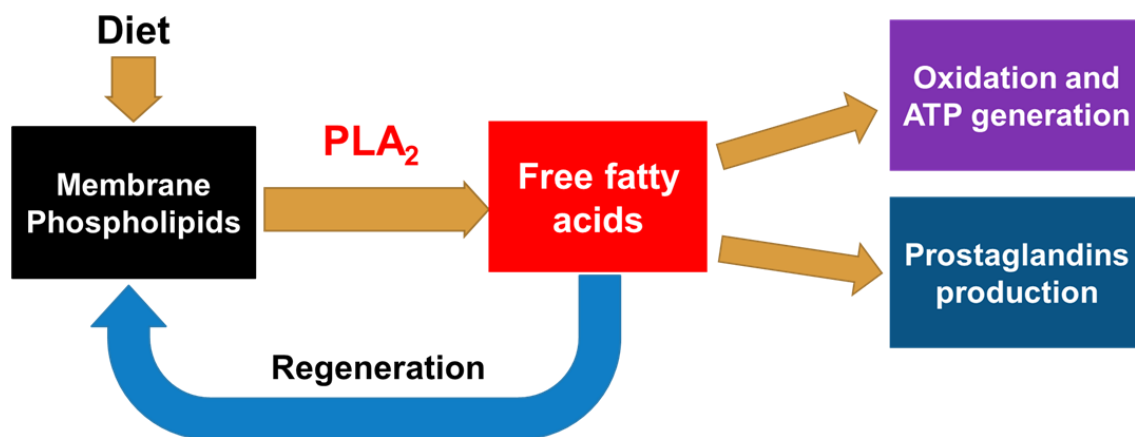


Figure 22. Lipolysis and FFAs' functions in lipid metabolism.

Abbreviations: PLA = phospholipase A2; ATP = Adenosine triphosphate.

5.2 EXPERIMENTAL PROCEDURES

5.2.1 Clinical design

FEANS and controls have been recruited from University of Pittsburgh western psychiatric institute site. Details of recruitment protocols have been fully illustrated in section 2.2.1. None of the subjects were on anticoagulants or lipid lowering agents before or during the study.

5.2.2 Gas chromatography analysis

Plasma FFAs and FAs bound on RBCs were quantitatively determined by capillary gas chromatography (GC) according to the procedure essentially the same as described by previous studies from Lepage (Lepage et al. 1988) and Ichihara (Ichihara et al. 1996), respectively. In brief, FFAs were extracted from 50 μ l of plasma containing internal standard (Tridecanoic acid) and converted to methyl esters by acetyl chloride-methanol reagent. RBC FAs were extracted from 150 μ l of RBC samples containing internal standard (Diheptadecanoyl lecithin) and converted to methyl esters by methanolic KOH reagent. Then 1 μ l of resulting fatty acid methyl esters was injected for GC analysis. Each sample was tested in duplicates and QCs were used to check the differences between batches. The procedure used for GC analysis has been described elsewhere (Reddy et al., 2004). Each sample was run under a splitless injection mode with helium as the carrier gas (3 mL/minute) and with an inlet pressure of 6.5 psi. Linear standard curve range of this assay is from 0.25 nmol/mL to 1250 nmol/mL. The Lower Limit

of Quantification is 0.05 nmol/mL. Intra assay percentage of CV is below 5.0% and inter assay percentage of CV is below 6.5%. Peaks on the chromatograms were identified by comparing the retention times with those of standard mixtures (Supelco) and were calculated by Agilent ChemStation.

5.2.3 Cholesterol data collection

RBC membrane cholesterol levels in subjects were collected through hospital clinical sheets.

5.2.4 Data analysis

The ANOVA test was used to test differences between FFAs levels among HC, DC and FEANS groups. Post-Hoc tests were corrected by Bonferroni corrections. Sample T-test was used to test differences between levels of esterified FAs and cholesterol in RBC samples. Pearson correlation coefficients were used to test the associations of analytes. Regression analysis was conducted and adjusted by Bonferroni corrections. All tests were two-tailed.

5.3 RESULTS

5.3.1 Comparisons of total plasma FFAs, RBC cholesterol and esterified FAs levels between FEANS and HC groups

As shown in figure 22, significantly lower membrane-bound FAs and FFAs levels were observed in FEANS group in comparison with those in HC group. No significance difference of membrane cholesterol levels was found between FEANS and HC groups.

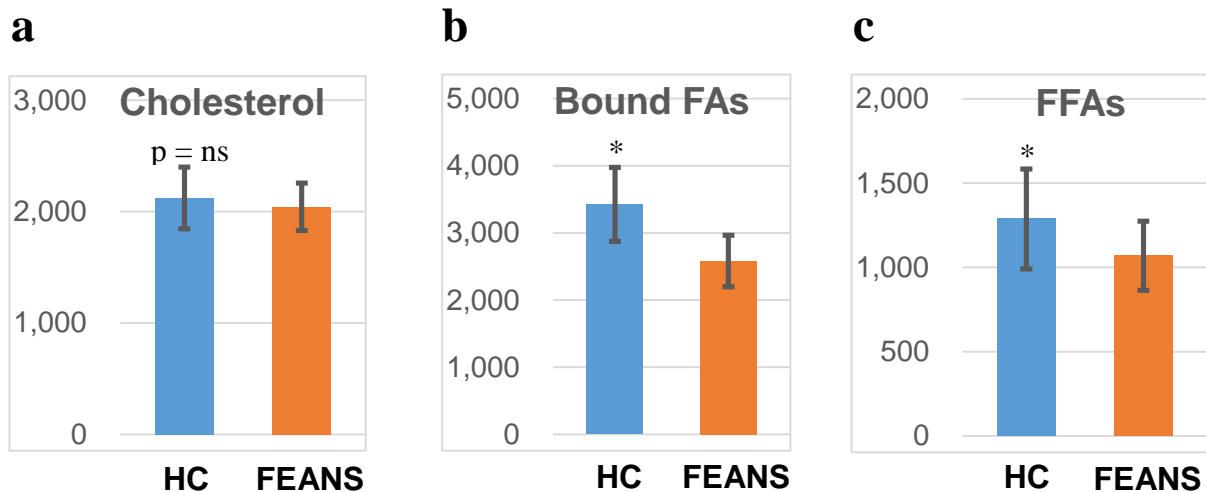


Figure 23. Comparisons of (a) cholesterol, (b) bound fatty acids and (c) free fatty acids levels between HC and FEANS groups.

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve SZ patients. All units are in nmol/mL. *, $p < 0.0001$.

5.3.2 Comparisons of FFA profiles among FEANS, DC and HC groups

We then compared the amount of plasma FFAs among FEANS, DC and HC groups. First, we observed a significantly decreased total levels of FFAs in FEANS group when comparing with

HC and DC groups. In consistent with the finding of decreased total FFAs levels in FEANS patients, we observed significant reduction of plasma FFAs mostly in saturated and n-6 fatty acid families (Table 12). Among saturated fatty acids, 16:0 is significantly decreased in FEANS group than in HC and DC groups. Among n-6 family fatty acids, 18:2n6c and 20:4n6 levels are significantly lower in FEANS group than in HC and DC groups.

Table 12. Table of fatty acid amounts among HC, DC and FEANS groups

Fatty acid ^a	HC	DC	FEANS	p₁^b	p₂	p₃
Saturates						
14:0	35 ± 16	29 ± 12	34 ± 20	ns	ns	ns
15:0	4.5 ± 1.8	5.9 ± 1.6	3.9 ± 1.9	ns	ns	ns
16:0	357 ± 95	356 ± 82	295 ± 74	ns	0.0007	0.007
17:0	6.6 ± 4.5	6.6 ± 2.7	4.9 ± 1.8	ns	ns	ns
18:0	109 ± 30	125 ± 28	98 ± 21	ns	ns	0.0004
21:0	5.1 ± 3.6	6.5 ± 2.5	4.5 ± 3.6	ns	ns	ns
22:0	11.2 ± 4.7	12.6 ± 4.5	9.0 ± 3.2	ns	ns	ns
Monoenes						
16:1n7t	5.8 ± 2.4	6.9 ± 2.3	4.5 ± 1.6	ns	ns	<0.0001
16:1n7c	22.6 ± 10.7	26.7 ± 11.2	17.1 ± 7.8	ns	ns	0.0003
17:1	9.2 ± 7.0	12.2 ± 4.1	7.8 ± 5.3	ns	ns	0.014
18:1n9t	25 ± 14	27 ± 11	21 ± 12	ns	ns	ns
18:1n9c	227 ± 73	213 ± 54	181 ± 46	ns	0.001	ns
18:1n7	25 ± 10	28 ± 8	22 ± 8	ns	ns	ns
Dienes						
18:2n6c	314 ± 75	322 ± 65	255 ± 62	ns	<0.0001	<0.0001
20:2n6	5.1 ± 3.6	5.1 ± 2.3	3.6 ± 2.4	ns	0.013	ns
Trienes						
18:3n6	5.6 ± 2.7	5.3 ± 1.9	4.6 ± 2.1	ns	ns	ns
20:3n6	5.3 ± 2.9	5.3 ± 2.3	4.3 ± 2.8	ns	ns	ns
Tetraenes						
20:4n6..	70 ± 17	75 ± 10	60 ± 16	ns	0.004	0.0004
22:4n6..	13.4 ± 4.6	15.4 ± 4.1	12.9 ± 4.5	ns	ns	ns
Pentanenes						
22:5n3..	5.0 ± 2.6	5.7 ± 2.2	3.9 ± 2.7	ns	ns	ns
Hexananes						
22:6n3..	26 ± 9	25 ± 7	22 ± 3	ns	0.004	ns
Totals						
Saturates	529 ± 129	542 ± 118	450 ± 91	ns	0.001	0.002
n-3	31 ± 10	31 ± 8	26 ± 5	ns	0.006	ns
n-6	413 ± 90	428 ± 65	340 ± 74	ns	<0.0001	<0.0001
Totals	1288 ± 297	1315 ± 213	1069 ± 205	ns	<0.0001	0.0003

^a All amounts are expressed as mean ± standard deviation. Units: nmol/mL. ^bp₁: HC group vs DC group. p₂: HC group vs FEANS group. p₃: DC group vs FEANS group. Critical p value after Bonferroni correction: p=0.017.

Disrupted correlations within fatty acid families were also observed in FEANS group but not in HC or DC groups. While significant correlations were still observed in saturated fatty acid family (Figure 23) among all three groups, significant correlations between 17:0 and 17:1 as well as 18:3n6 and 20:3n6 were found only in HC and DC subjects but not in FEANS subjects (Figure 24).

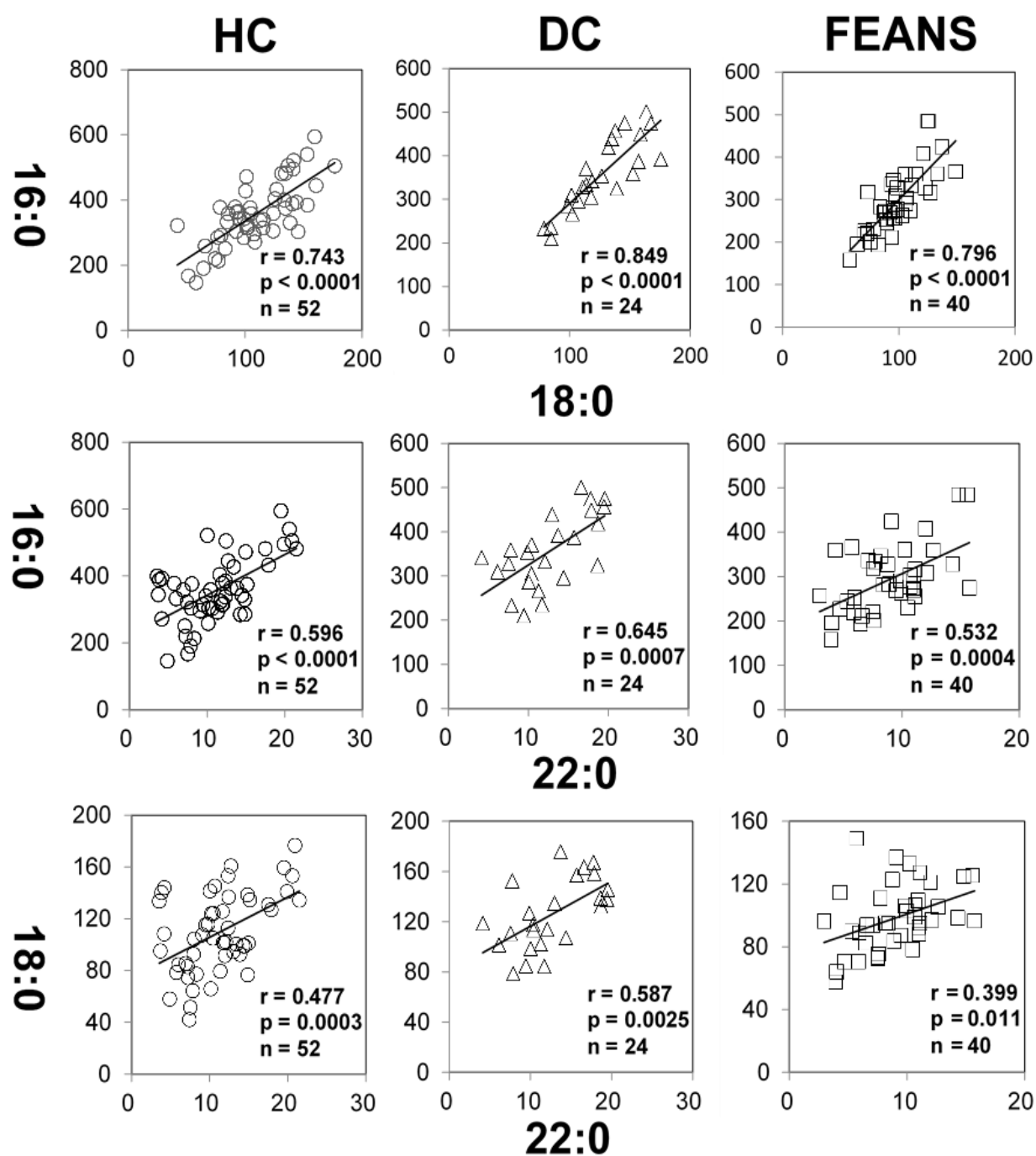


Figure 24. Comparisons of correlations between precursor and product fatty acids among HC, DC and FEANS groups

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve SZ patients. All units are in nmol/mL. Critical p value after Bonferroni correction: 0.017.

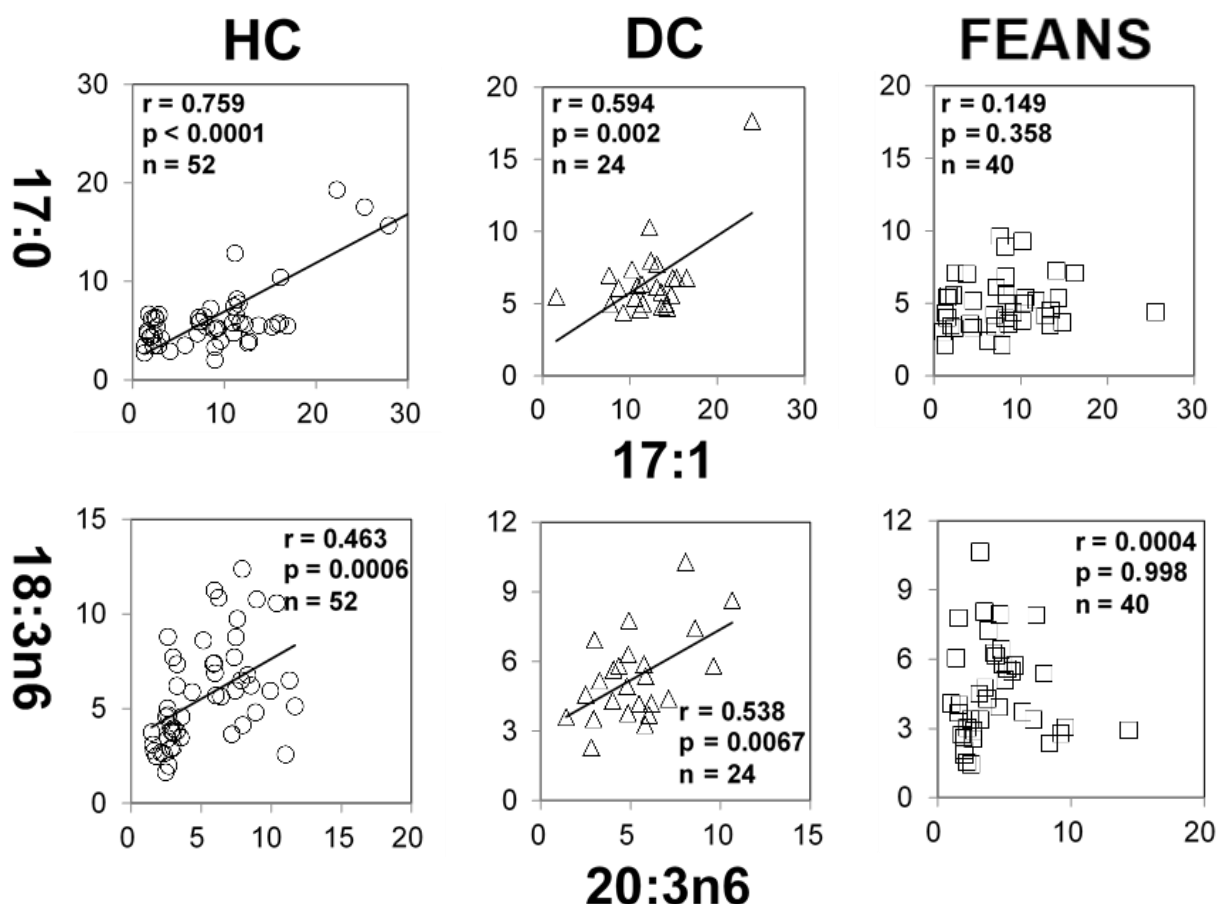


Figure 25. Comparisons of correlations between precursor and product fatty acids among HC, DC and FEANS groups.

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve SZ patients. All units are in nmol/mL. Critical p value after Bonferroni correction: 0.017.

5.3.3 Correlations between adenosine and FA levels in HC and FEANS groups

In section 5.3.1, significantly decreased levels of membrane-bound FAs and plasma FFAs were observed in FEANS group when comparing with those in HC and DC groups. Here we tested

if such alterations correlate with adenosine levels. Results in figure 25 showed that in both HC and FEANS groups, adenosine levels significantly correlate with membrane-bound FAs but not with FFAs.

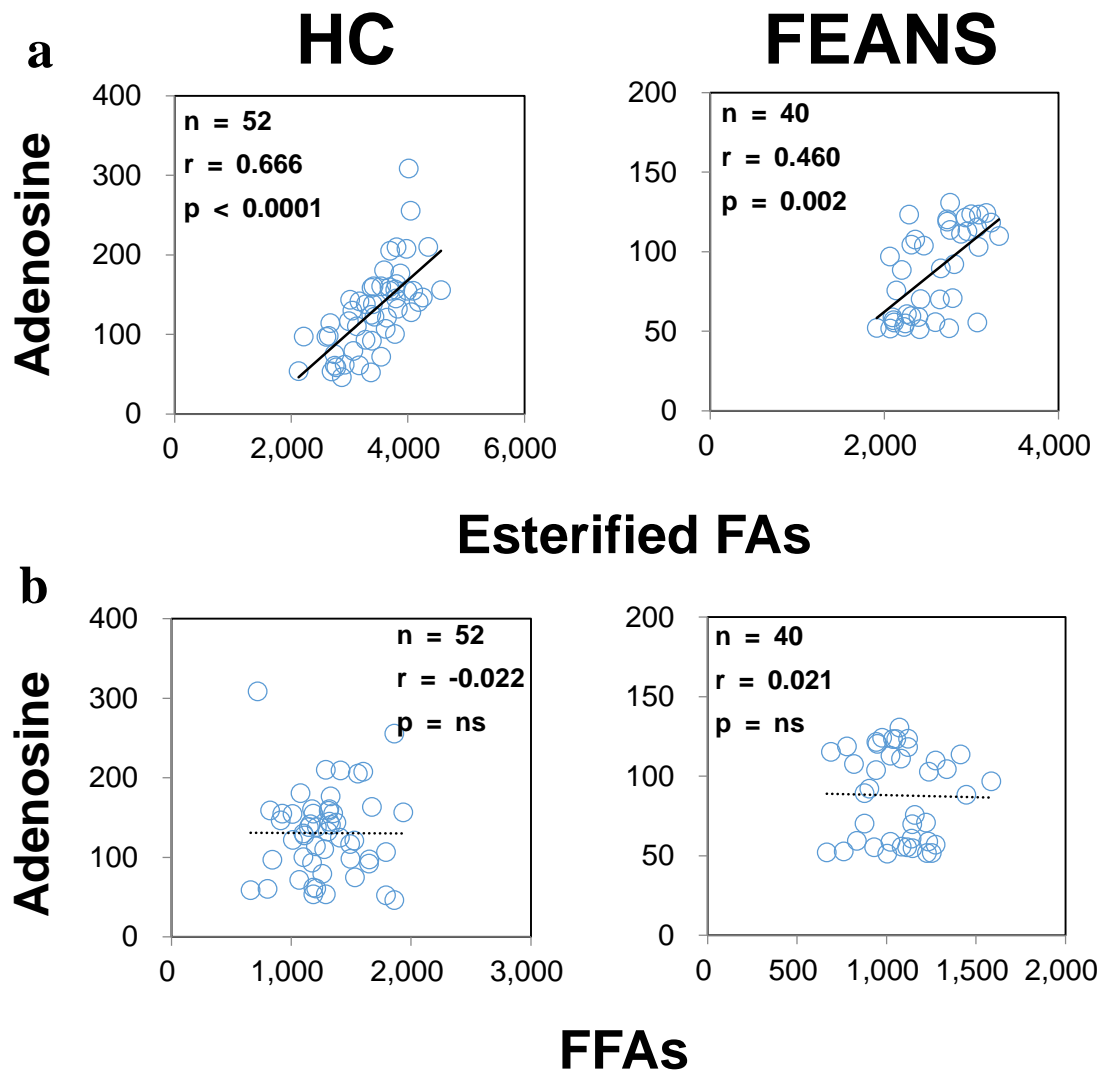


Figure 26. Correlations between adenosine and (a) esterified FAs and (b) FFAs in HC and FEANS groups.

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve SZ patients. All units are in nmol/mL. Critical p value after Bonferroni correction: 0.017.

5.4 DISCUSSION

In this chapter, we mainly compared RBC cholesterol, membrane-bound (esterified) FAs and plasma FFAs levels between HC and FEANS groups. We did not find any significant change of cholesterol levels in FEANS RBC samples when compared to levels in HC RBC samples. This finding indicated that cholesterol may not play a role in the membrane phospholipid alterations in SZ. Significantly decreased levels of membrane-bound FAs were found in FEANS group in comparison to HC group. It corroborated the lipid theory of SZ pathology by suggesting an increased lipolysis on RBC cell membranes in SZ patients. Increased lipolysis of cell membranes and underlying clinical significance in SZ had been well-documented (Gattaz et al. 1990, Pettegrew et al. 1991, Horrobin et al. 1991 and Nojonen et al. 1993) in previous publications.

The resulting FFAs after membrane lipids lipolysis, however, had been scarcely studied in the pathology of SZ. Since it could help us to understand the metabolism of membrane lipids after the lipolysis, we measured FFAs levels and calculated compositions of the FFAs profiles in FEANS group and compared them with those in control groups. Instead of observing increased FFAs from increased breakdown of membrane phospholipids, we found a significant lower levels of FFAs in FEANS patients than in control subjects. These findings may suggest three important implications with regard to SZ pathology. First, reduced total FFAs levels may imply an increased regeneration of cell membranes. Reduced levels of plasma FFAs may reflect a depleted pool of those fatty acids resulting from an increased demand of cell

membrane regeneration during the early course of SZ development. Notably, while there is a general trend of decrease among all families of FFAs, we found that n-6 and saturated family FFAs levels in FEANS group are significantly lower than HC and DC groups. It may imply that these two FA families are the most vulnerable ones that been altered at the early stage of SZ.

Second, our findings reflected the disturbance of FA biosynthesis and following beta-oxidations at the early course of SZ. Most FFAs are synthesized by elongases & desaturases, and then go through beta-oxidation to generate energy (Figure 25). Although some of precursor to product correlations persist across disease and normal status, others appear to be lost in FEANS group. Results suggested that the potential for steady formation of fatty acids is altered early in the course of illness. While alterations were also observed in saturated fatty acid family, it is the n-6 fatty acid family that seems to be most altered in FEANS subjects. This finding suggested that n-6 fatty acid biosynthesis are more disrupted than other fatty acid families at the early course of the disease. Furthermore, previous studies also showed that several fatty acid beta-oxidation enzymes were significantly increased in SZ patient brain, which indicates an enhanced beta-oxidation in FEANS patients (Prabakaran et al. 2004). Yang et al (2017) also suggested an up-regulated beta-oxidation process in the SZ patient serum by comparing product to precursor ratios of FAs. Therefore, we hypothesize that the increased lipolysis of membrane lipids and the reduction of following plasma FFAs may due to the hyperactivity of beta-oxidation in SZ pathology.

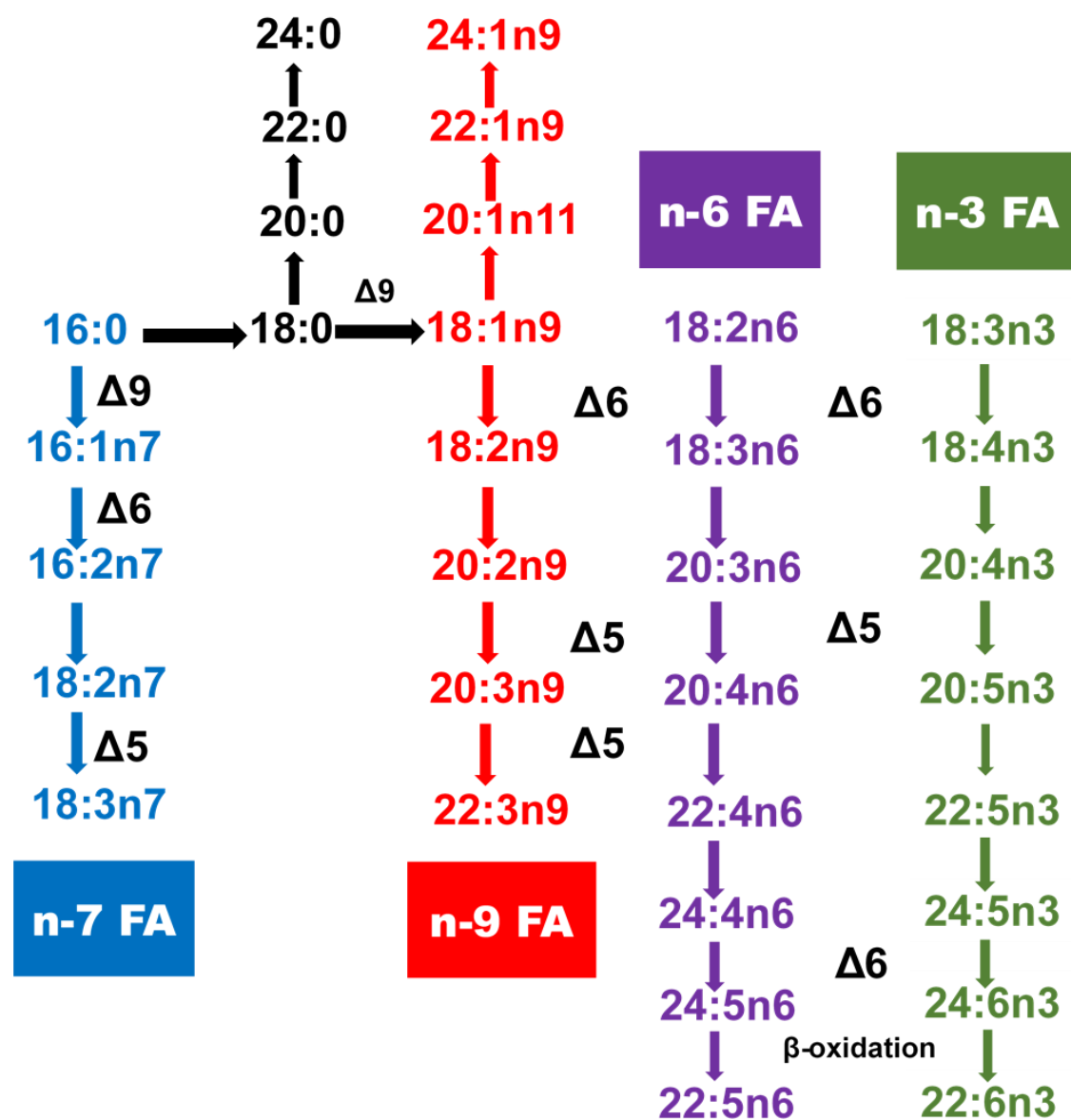


Figure 27. Fatty acid metabolic pathways.

Abbreviation: FA = fatty acid; Δ = desaturase. Different families are indicated by different colors.

Third, findings may help to explain the blunted niacin-induced skin flushing in a subgroup of SZ patients. Niacin test refers to the evaluation of aqueous methyl nicotinate's effect on the production of prostaglandins and the following cutaneous capillary vasodilatation. The mechanism of the niacin response has been illustrated by previous literatures (Messamore and Yao, 2016). In normal condition, niacin induces flushing response mediated through niacin

receptors, and activates PLA₂ to release arachidonic acid (Tang et al. 2006). Released free arachidonic acid is the substrate of vasodilatory prostaglandins (PGD₂ and PGF₂) and these prostaglandins activates prostanoid receptors to dilate cutaneous blood vessel (Lai et al. 2007) and eventually lead to skin flush (Benyó et al. 2005, Maciejewski-Lenoir et al. 2006, Morrow et al. 1989, Morrow et al. 1992). It is interesting to note that blunted niacin-induced skin flushing was found in a subgroup of SZ patients. Previous literature pointed out that in aqueous methyl nicotinate test on skin, schizophrenics were highly significantly different from the controls among all concentrations of aqueous methyl nicotinate (Ward et al. 1998). Moreover, at certain concentration, 83% of schizophrenics but only 23% of controls were having a zero or minimal response to aqueous methyl nicotinate (Ward et al. 1998). This study was further replicated by Puri et al on 2001 and suggested that this test may be useful clinically in the diagnosis of SZ (Puri et al. 2001). While the cause of the blunted skin flushing in SZ is still unclear, our findings of significant decreased arachidonic acid (20:4n6) as well as its precursors in FEANS patients may offer a possible explanation for the underlying mechanism. Based on our findings, it is speculated that reduced levels of 18:2n6 and 20:4n6 may reflect a decreased pool for subsequent production of prostaglandin vasodilators, which leads to the blunted niacin-induced flushing response in those SZ patients.

In this chapter, we are interested in learning adenosine's effect on membrane lipid lipolysis in SZ. According to previous findings, activation of A₁R can inhibit lipolysis through enhancing insulin's inhibitory effect on the activity of phospholipase (Schwabe et al. 1974, Johansson et al. 2008). The mechanism by which adenosine enhances insulin's effect is

mediated by the activation of cAMP-dependent protein kinase (Koupenova et al. 2013). As expected, our results demonstrated a significant correlation between adenosine and membrane bounded total FA levels. Since in chapter 2, reduced adenosine levels were observed and adenosine signaling deficits were suggested in SZ patients, thus it is speculated that the increased membrane lipolysis and reduced levels of membrane FAs may result from a reduced adenosinergic activity in SZ. However, no significant correlation was observed between total FFAs levels and adenosine in either HC or FEANS group. This finding might be due to the multiple sources that producing FFAs in the plasma. Besides originated from membrane phospholipids, plasma FFAs can also be produced from the lipolysis of triglycerides and lipoproteins (Wang et al. 2009, Teusink et al. 2003). Also, since plasma FFAs served as a dynamic circulating pool of FAs, its total levels can also be affected by multiple downstream biochemical reactions such as beta-oxidation and prostaglandin biosynthesis. More research are needed to investigate plasma FFAs' origins and its metabolisms in SZ.

5.5 CONCLUSION

1. In consistent with lipid theory of SZ, significantly reduced membrane-bound FAs' levels were observed in SZ patients in comparison to HC subjects, implying an increased lipolysis of membrane phospholipids in SZ pathology. The analysis of plasma FFAs' levels and lipid compositions also yield interesting results. Specifically, we observed a general trend of FFAs' level reduction and biosynthesis disturbance in FEANS group. We also associated these

metabolic dysfunctions with clinical implications with regard to SZ pathology.

2. We found significant correlation between adenosine and membrane FAs in both HC and FEANS groups, suggesting that adenosine level reductions might contribute to membrane phospholipid defects in SZ pathology.

To summarize, based on all information gathered, a hypothesis was made that adenosine signaling deficits were at least partially contributed to membrane phospholipid defects in SZ. Specifically, since adenosine signaling was reduced in SZ, adenosine's up-regulating effect on insulin activity through cAMP-dependent protein kinase pathway was also diminished. As insulin activity was decreased, the inhibitory effect of insulin on membrane PLA₂ activity through phosphoinositide 3-kinases pathway thus was weakened. Consequently, lipolysis activity through membrane PLA₂ was increased and finally resulted in membrane phospholipids defects in SZ which has been illustrated in the lipid theory. However, more evidences with regard to adenosine's role in lipolysis are still in need to validate this hypothesis.

6.0 ASSOCIATIONS OF ADENOSINE SIGNALING DEFECTS AND DOPAMINE

METABOLIC DEFECTS IN SCHIZOPHRENIA

6.1 BACKGROUND

Dopamine theory states that it is the increased dopaminergic signaling in striatal region that leads to SZ symptoms. Adenosine's role in regulating dopaminergic signaling in the striatum and findings of dopamine metabolism alterations have been summarized in section 1.5. Thus we tested if those alterations of dopaminergic signaling can also be found in the FEANS group when comparing with HC group, and if so, whether such alterations could be linked to adenosine levels or not.

This chapter also serves as a part of the aim 3 in the dissertation. We tested two sub-aims in this chapter. Results & discussion are based on the flow of following sub-aims.

Aim 3.3.a. Test if there is a hyperactivity of dopamine metabolism in the SZ patient plasma. As is shown in figure 27, dopamine is synthesized from phenylalanine (Phe) and is eventually metabolized to homovanillic acid (HVA). Because of the extremely low levels (less than 30 pg/mL) of dopamine, 3-methoxytyramine and 3, 4-dihydroxyphenylacetic acid in plasma, we measured levels of Phe, tyrosine (Tyr) and HVA to indirectly reflect dopamine metabolism in the peripheral tissues of SZ patients. Previous findings about Phe and Tyr plasma levels in SZ have been summarized in table 2. HVA is the final product of dopamine metabolism. Plasma HVA levels is often used to predict striatal dopaminergic activity (Pickar

et al. 1988, Beuger et al. 1996) and treatment outcomes (Mazure et al. 1991, Davila et al. 1988). Since dopamine theory suggested a hyperfunction of striatal dopamine metabolism in SZ patients, we are predicting higher levels of Phe, Tyr and HVA in the FEANS group than those in the HC group.

Aim 3.3.b. Test if reduced adenosine levels link to the dopamine metabolic defects in SZ. In chapter 2, significantly decreased plasma adenosine levels were observed in the FEANS group when comparing with those in control groups. In this aim, we will test if adenosine levels correlate with analytes' levels in the dopamine metabolism.

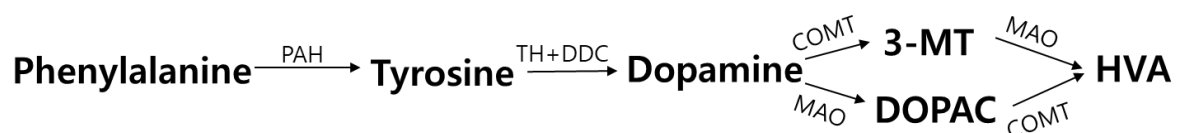


Figure 28. Dopamine metabolic pathway.

Abbreviations: PAH = phenylalanine hydroxylase; TH = tyrosine hydroxylase; DDC = dopa decarboxylase; COMT = catechol-O-methyltransferase; MAO = monoamine oxidase; 3-MT = 3-methoxytyramine; DOPAC = 3, 4-dihydroxyphenylacetic acid; HVA = homovanillic acid.

6.2 EXPERIMENTAL PROCEDURES

6.2.1 Clinical design

Subjects were recruited through the protocol that has been illustrated in section 2.2.1. Samples were collected from forty FEANS patients and fifty-two age-, gender-, BMI- and race-matched

HC subjects. Sample graphic table is the same as shown in table 3. Samples from DC group were not involved in this study.

6.2.2 Gas chromatography analysis

Phe and Tyr levels in plasma were quantitatively determined by GC. Samples were prepared according to the instruction of amino acid analysis kit from Phenomenex (AG0-7184). Briefly, 50 μ L of internal standard (Norvaline at 200 μ M) was added into each 100 μ L plasma sample and samples were then transferred into solid-phase extraction tube. Organic derivatizing reagent (2,2-dihydroxyindane-1,3-dione) was then added into the tube. At last, amino acids were extracted in organic phase and 1 μ L of each sample was injected into the GC system. Each sample was tested in duplicates and QCs were used to check the differences between batches. Each sample was run under a splitless injection mode with helium as the carrier gas (1.5 mL/minute) and with an inlet pressure of 6 psi. Peaks on the chromatograms were identified by comparing the retention times with those of standard mixtures (EZfaast) and were calculated by Agilent ChemStation. Linear standard curve range of this assay is from 5 μ M to 400 μ M. The Lower Limit of Quantification is 0.1 μ M. Intra assay percentage of CV is below 5.0% and inter assay percentage of CV is below 5.5%.

6.2.3 High-pressure liquid chromatography assay

HVA was determined by the HPLC-CEMAS. Protocol and procedures of the assay have been illustrated in section 4.2.2. Linear standard curve range of this assay is from 2.5 ng/mL to 100 µg/mL. The Lower Limit of Quantification is 1.0 ng/mL. Intra assay percentage of CV is below 1.5% and inter assay percentage of CV is below 2.0%.

6.2.4 Data analysis

Sample T-test was used to test differences of analytes' levels between FEANS and HC groups. Pearson correlation coefficients were used to test the associations of analytes. Regression analysis was conducted and corrected with Bonferroni adjustment. All tests were two-tailed.

6.3 RESULTS

6.3.1 Comparisons of plasma analytes' levels in dopamine metabolism between FEANS and HC groups

Phe and Tyr are important precursors in the biosynthesis of dopamine. Thus we first compared these two amino acids' levels in the plasma between FEANS and HC groups. As expected, results showed significantly higher levels of Phe and Tyr in FEANS group than those in the HC group (Figure 28). We also compared levels of the end product of dopamine metabolism (i.e. homovanillic acid) in the plasma between FEANS and HC groups. Plasma HVA levels in the FEANS group were also observed that were significantly higher than those in the HC group (Figure 28).

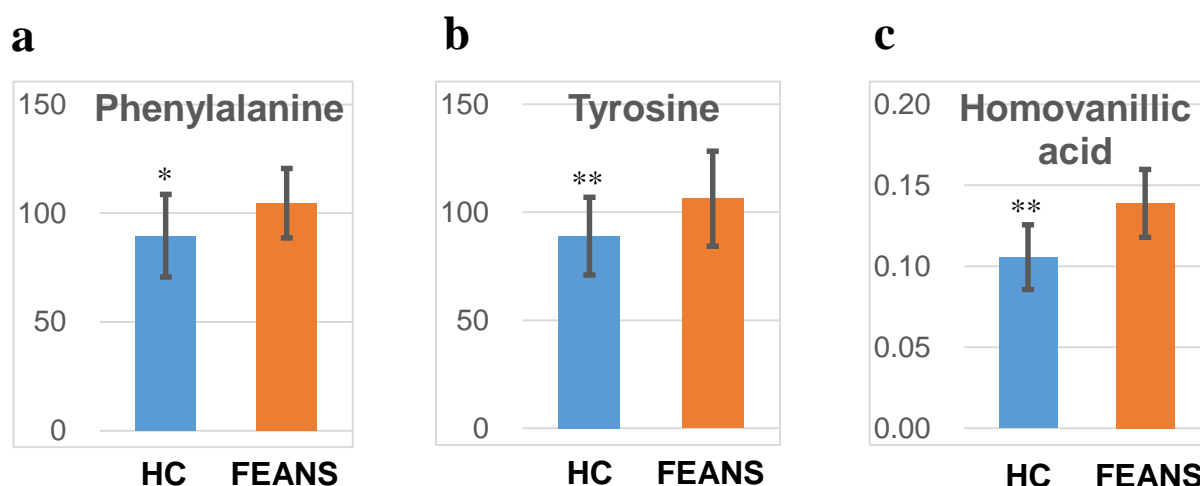
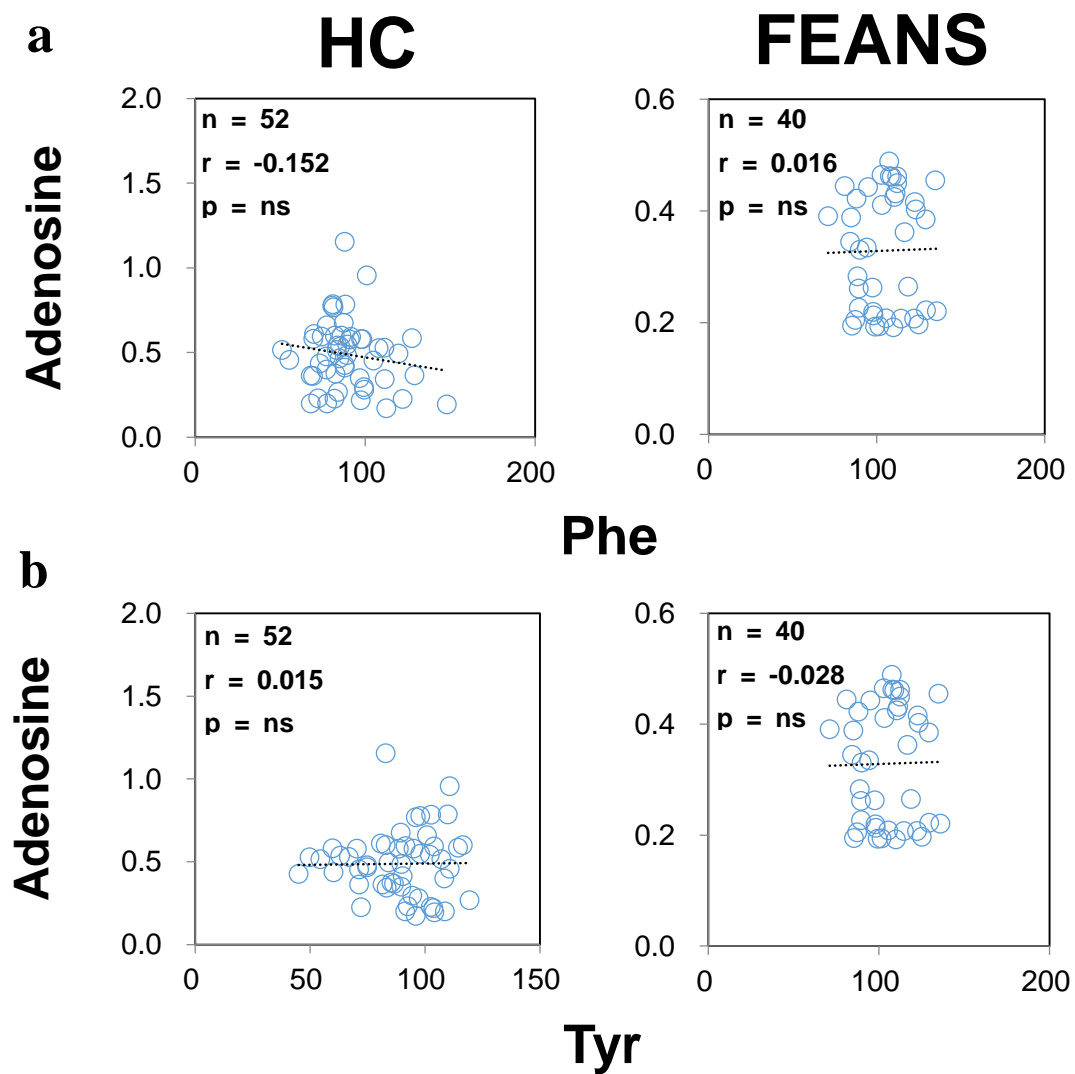


Figure 29. Comparisons of (a) phenylalanine, (b) tyrosine and (c) homovanillic acid levels between HC and FEANS groups.

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve schizophrenia patients. All units are in μM . *, $p < 0.001$; **, $p < 0.0001$.

6.3.2 Correlations between adenosine and analytes in the dopamine metabolism in HC and FEANS groups

We then tested correlations between adenosine and analytes in the dopamine metabolism. Interestingly, we didn't observe significant correlation between adenosine and phenylalanine or between adenosine and tyrosine in either FEANS group or HC group (Figure 28). However, we found significant inverse correlations between adenosine and HVA in both groups (Figure 28).



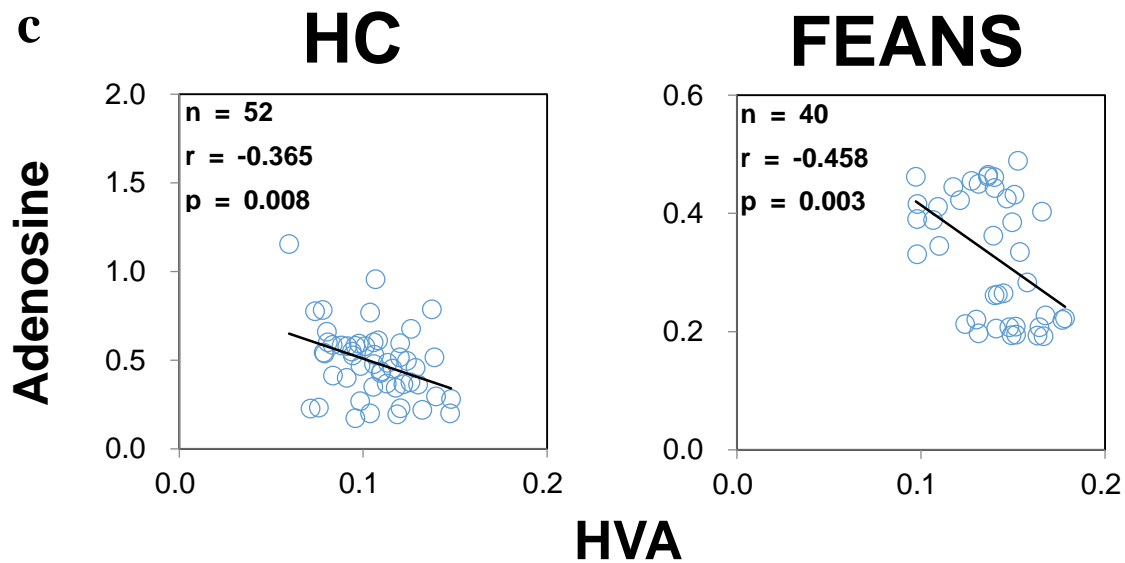


Figure 30. Correlations between adenosine and (a) phenylalanine, (b) tyrosine and (c) homovanillic acid in HC group and FEANS group.

Abbreviations: HC = healthy controls; FEANS = first-episode antipsychotic naïve schizophrenia patients; Phe = phenylalanine; Tyr = Tyrosine; HVA = homovanillic acid. All units are in μM . Critical p value after Bonferroni correction: 0.00083.

6.4 DISCUSSION

In this chapter, we have determined plasma levels of Phe, Tyr and HVA in FEANS and HC groups. Significantly increased Phe, Tyr and HVA levels were observed in FEANS group when comparing with HC group. Data here supported those previous findings that reported a hyperfunction of dopaminergic activity in the peripheral tissues of SZ patients which linked to striatal dopamine hyperfunctions in SZ (Howes et al. 2009, Rao et al. 1992, Sumiyoshi et al. 2000). Furthermore, our findings suggested that both the biosynthesis and the metabolism of dopamine have been increased at the early course of SZ. However, since we were not able to measure dopamine and its two direct metabolites (3-methoxytyramine and 3, 4-

dihydroxyphenylacetic acid) in this dissertation, the mechanism of the increased biosynthesis of dopamine that leads to increased levels of the final product is still not clear. In other words, whether both of the dopamine direct turnovers' levels have been altered by SZ remains unknown at current stage and this issue should be noted as a limitation of our present study. More research are required to investigate whether increased plasma levels of HVA were resulted from increased levels of both of the dopamine direct metabolites (3-methoxytyramine and 3, 4-dihydroxyphenylacetic acid) or from one of them.

To test adenosine's effect on dopamine metabolism, we correlated adenosine with metabolites in dopaminergic signaling in both FEANS and HC groups. No significant correlations between Phe and adenosine or between Tyr and adenosine were observed. This finding implied that adenosine may not have an impact on the biosynthesis of the dopamine. On the other hand, the significant inverse correlation between HVA and adenosine were observed, which suggested that adenosine may have an inhibitory effect on dopaminergic signaling after dopamine is synthesized. Previous findings from animal models also implied that adenosine mainly inhibit dopaminergic signaling through ARs-DRs interactions (Ferré et al. 1997, Ferré et al. 2008, Le Moine et al. 1997). According to results, we speculated that such inhibitory effect was not altered by SZ, as we found significant inverse correlations between adenosine and HVA in both FEANS and HC groups. Instead, it is possible that it is the decreased levels of adenosine that contributed to the abnormal dopaminergic signaling in SZ.

6.5 CONCLUSION

1. Significantly increased dopaminergic signaling metabolites' levels in FEANS group were observed when comparing to HC group, suggesting a hyperfunction of dopaminergic signaling in peripheral tissues at the early course of the SZ.
2. Adenosine seems cannot affect the biosynthesis of dopamine. Instead, levels of the end product in dopamine metabolism (HVA) were observed that inversely correlate with adenosine levels, which supported adenosine theory in that adenosine could inhibit the dopaminergic signaling in peripheral tissues at least partially via ARs-DRs interactions.

In conclusion, adenosine signaling deficits might play an important role in the hyperfunction of dopaminergic signaling that have been well-documented in the SZ pathophysiology. Specifically, it is speculated that decreased adenosine levels may cause the decreased inhibitory effect of adenosine on dopaminergic signaling via ARs-DRs interactions. The hyperfunction of dopaminergic signaling then leads to the onset of clinical symptoms especially positive ones in the SZ symptomatology.

7.0 CLINICAL ASSOCIATIONS OF ADENOSINE SIGNALING AND SCHIZOPHRENIA SYMPTOMATOLOGY

7.1 BACKGROUND

In the United States, SZ is diagnosed based on the criteria in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM). First, to be diagnosed with SZ by DSM IV (Fourth Edition. 1994, 2007), two of the following characteristic symptoms need to be met over at least one month: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior or negative symptoms (e.g. flattened affect, alogia, amotivation, avolition). Second, the person should have social or occupational dysfunction or failure to achieve a level of functioning expected for their age and socioeconomic background. Third, continuous signs of the disturbance must persist for a minimum of six months. Fourth, schizoaffective disorder and mood disorders have been ruled out (American Psychiatric Association 2007). For studies relevant to this dissertation, detailed symptom assessments and diagnostic assessments were fully reviewed (Andreasen et al. 1992, Andreasen et al. 1995) using the principles of CASH (comprehensive assessment of symptoms and history). Rating data used in this dissertation also meets with such standards.

The adenosine theory hypothesizes that adenosine signaling deficits will correlate with SZ symptoms. Specifically, it proposes that adenosine signaling deficits correlate positively with positive symptoms via DRs-ARs interactions and correlate positively with negative &

cognitive symptoms via NMDARs-ARs interactions (Lara et al. 2006, Boison et al. 2012). This assumption is based on two sources of supporting evidences. Firstly, as introduced in section 1.2, adenosine is the up-stream regulator of dopaminergic signaling (S. Ferré et al. 1991a, S. Ferré et al. 1991b, Salmi et al. 2005) and glutamatergic signaling (Boison. 2008, Okamura et al. 2004) via ARs. Secondly, striatal dopaminergic signaling hyperfunction has been mostly linked to positive symptoms (Davis & Kahn. 1991, Howes & Kapur. 2009) while NMDAR hypofunction has been linked to cognitive & negative symptoms (Coyle & Tsai. 2004, Farber. 2003) of SZ. However, since adenosine has been scarcely studied in psychiatry, few studies have directly correlated adenosine with SZ symptoms. In previous chapters, we have tested the presence of adenosine signaling deficits in SZ patients. We also found that adenosine signaling could be affected by antipsychotics and correlates to other metabolic defects in SZ. Here in this chapter, we will test whether adenosine signaling deficits correlate with SZ symptoms.

This chapter also serves as aim 4 in the dissertation. We correlated positive, negative and global symptom assessment with adenosine signaling in FEANS and chronic off-treatment SZ groups.

7.2 METHODS

7.2.1 Patients recruitment

1. **Chronic SZ group.** The recruitment procedure has been introduced in 2.2.1.
2. **FEANS group.** The recruitment procedure has been introduced in 2.2.1.

7.2.2 Adenosine, ADA and inosine data collection.

Biochemical data were collected using ELISA assay applied to clinical samples mentioned in 2.2.1. Details of experimental procedure have been revealed in 2.2.2.

7.2.3 Clinical evaluation

Data were collected from hospital records. Clinical interviews were conducted on each subject by a mental health professional. Brief Psychiatric Rating Scale (BPRS), Scale for the Assessment of Positive Symptoms (SAPS), Scale for the Assessment of Negative Symptoms (SANS) and Global Assessment of Functioning Score (GAS) were used with the FEANS group evaluation (Overall and Gorham. 1962). SAPS and SANS were used with the chronic on-/off-treatment SZ group. The BPRS contains 24 items that reflect a broad range of symptomatology, including mood symptoms (depressive and manic), and anxiety symptoms in addition to psychotic symptoms. For studies here, the positive (BPRS-P, including conceptual disorganization, suspiciousness, hallucinatory behavior and unusual thought content) and

negative (BPRS-N, including emotional withdrawal, motor retardation and blunted affect) symptom subscales (Ventura et al. 2000) were used. Each item focuses on one specific symptom (such as hallucinations, depressive mood, motor retardation, grandiosity, etc.). Each item ranges from 0 (no symptom) to 7 (most severe) to reflect the severity of each symptom. Total Scores were used for all correlation analyses. SAPS and SANS were also used to assess positive and negative symptoms, respectively. The SAPS has 34 items (including four global items) that constitute four subscales measuring hallucinations, delusions, bizarre behavior and positive formal thought disorder. The SANS has 25 items (including five global items) that constitutes five subscales measuring affective flattening or blunting, alogia, avolition-apathy, anhedonia-asociality and attention. Each item was also scored by clinicians from 0 (no symptom) to 5 (most severe) to evaluate the degree of each symptom. Total Scores were used for the correlation analysis. The GAS was used to rate how serious a mental illness may be. It measures severity of symptoms and how much a person's symptoms affect his or her daily life on a scale of 0 (profound negative effects on daily functioning) to 100 (no symptoms present and no deficits in daily functioning). Diagnoses were confirmed at diagnostic conferences attended by research faculty and staff, and chaired by an experienced psychiatrist.

7.2.4 Data analysis

Correlations between adenosine signaling and symptom assessment scales were tested in FEANS group and off-treatment SZ group via linear regressions. Bonferroni correction was applied in each of the regression. All tests were two-tailed.

7.3 RESULTS

7.3.1 Correlations between adenosine signaling and SZ symptoms in FEANS group

As hypothesized, Results showed significant correlations between adenosine signaling and positive symptoms. As shown in Figure 30, significantly inversed correlations were observed between adenosine and positive symptoms as measured on the BPRS and SAPS. Significant positive correlation was observed between ADA and BPRS positive symptoms. A significantly inversed correlation was observed between inosine and positive symptoms as measured on the SAPS. Also, significant correlations were found for adenosine and ADA with GAS scores. However, none of the negative symptom scales correlated with adenosine signaling (Figure 31).

7.3.2 Correlations between adenosine signaling and SZ symptoms in chronic off-treatment SZ group

As hypothesized, in this group, we found significant inverse correlations between adenosine and SAPS in both plasma and CSF. No correlation was observed between SAPS scores and ADA or inosine (Figure 32). However, SANS scores did not correlate with any of the analytes in the adenosine signaling (Figure 33).

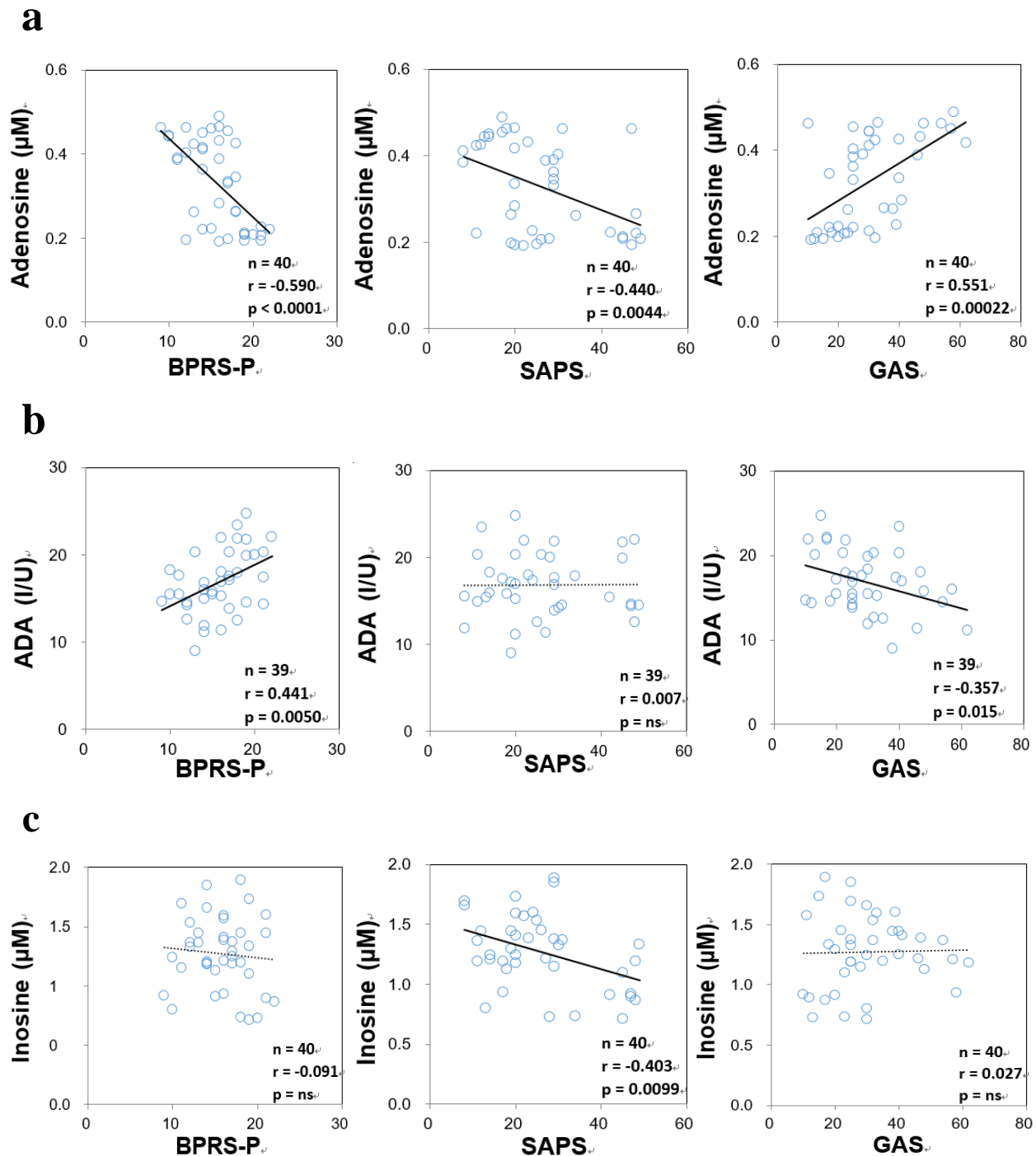


Figure 31. Correlations between symptoms scales and (a) adenosine, (b) ADA and (c) inosine.

Abbreviations: BPRS-P = brief psychiatric rating scale-positive symptoms; SAPS = scale for the assessment of positive symptoms; GAS = global assessment of functioning score. Critical p value after Bonferroni correction: 0.0167.

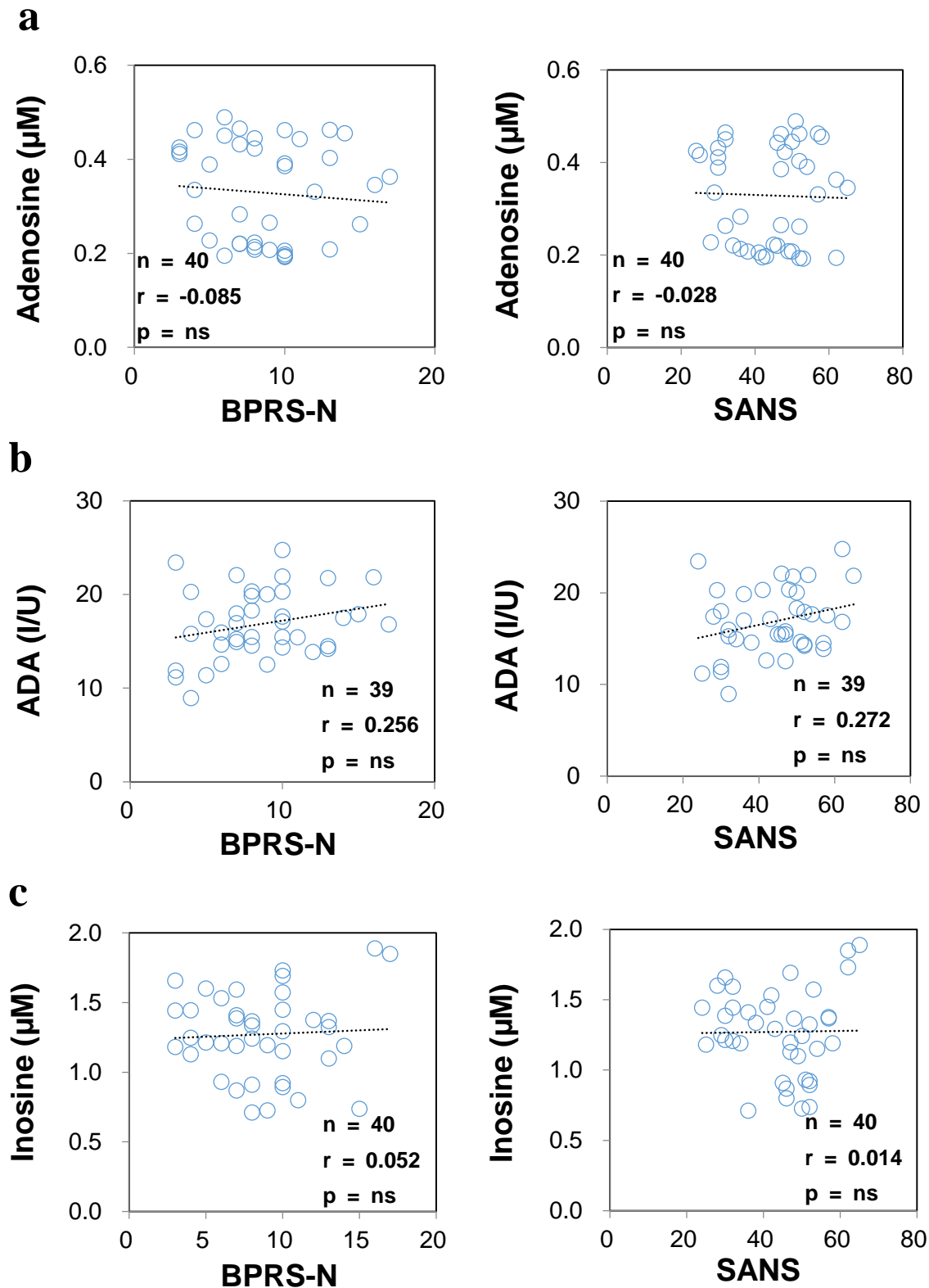


Figure 32. Correlations between symptoms scales and (a) adenosine, (b) ADA and (c) inosine.

Abbreviations: ADA = adenosine deaminase; BPRS-N = brief psychiatric rating scale-negative symptoms; SANS = scale for the assessment of negative symptoms. Critical p value after Bonferroni correction: 0.0167.

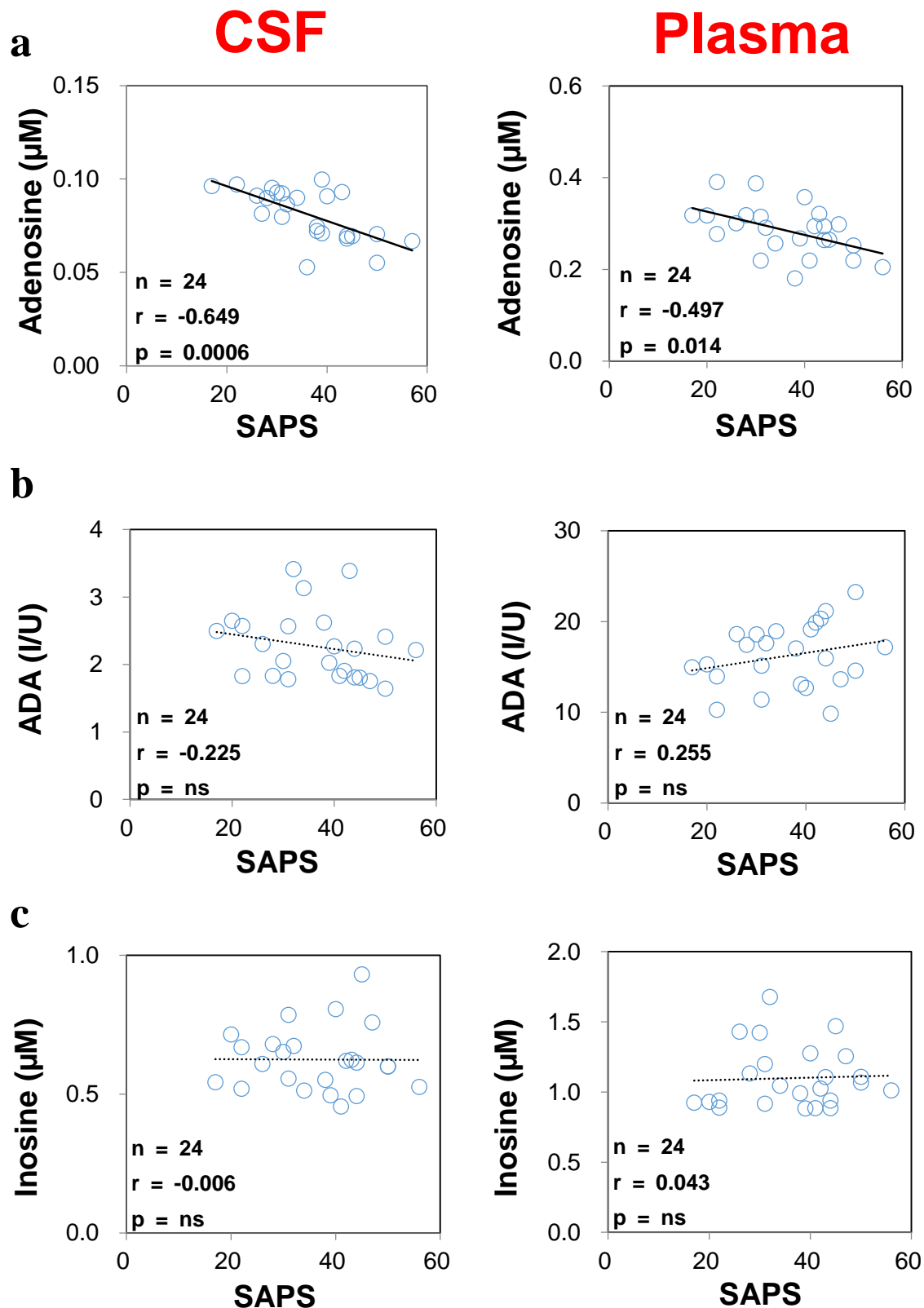


Figure 33. Correlations between positive symptoms and (a) adenosine, (b) ADA and (c) inosine in CSF and plasma.

Abbreviations: ADA = adenosine deaminase; CSF = cerebrospinal fluid; SAPS = scale for the assessment of positive symptoms. Critical p value after Bonferroni correction: 0.0167.

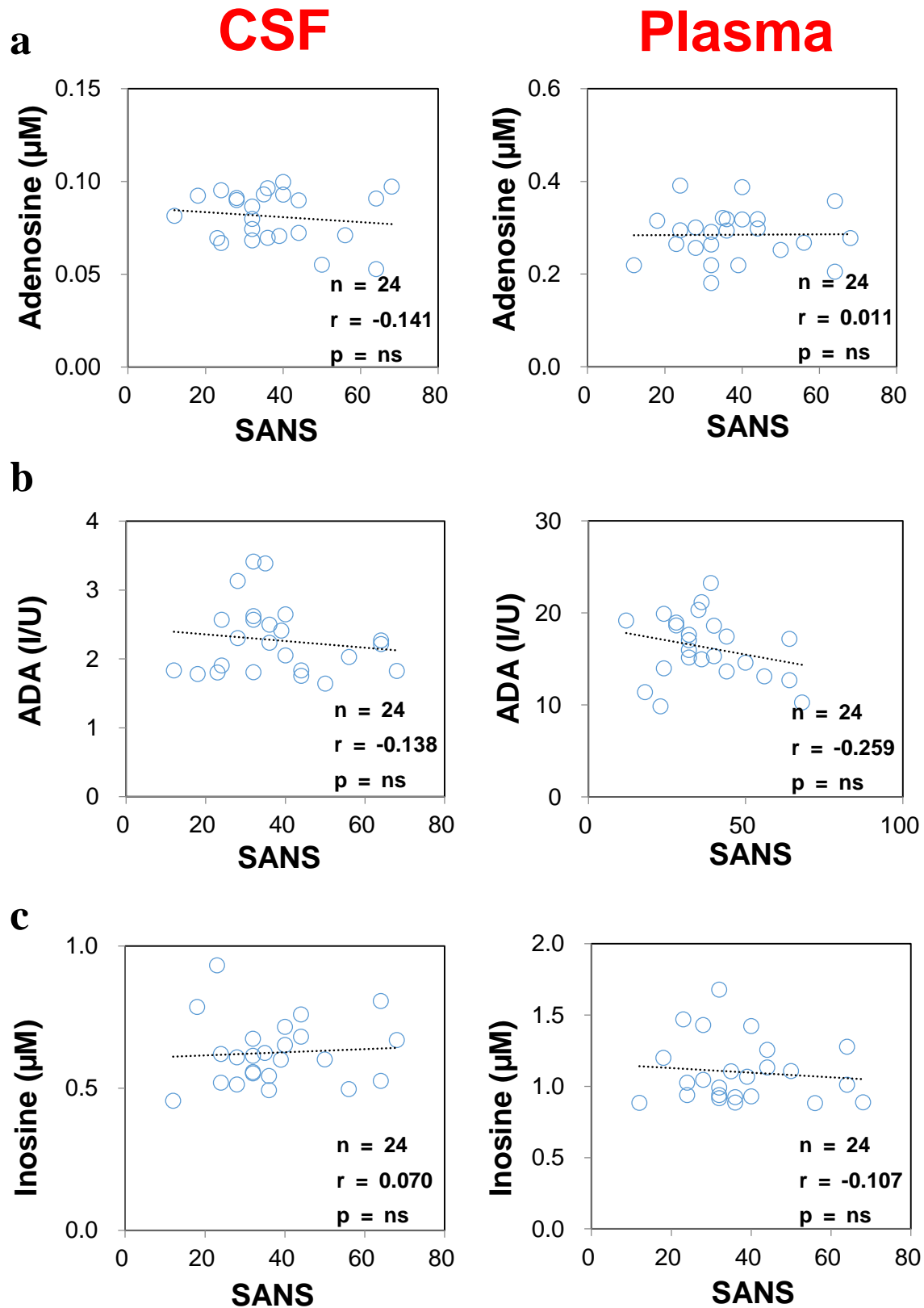


Figure 34. Correlations between negative symptoms and (a) adenosine, (b) ADA and (c) inosine in CSF and plasma.

Abbreviations: ADA = adenosine deaminase; CSF = cerebrospinal fluid; SANS = scale for the assessment of negative symptoms. Critical p value after Bonferroni correction: 0.0167.

7.4 DISCUSSION

In this chapter, we tested the relationship between the adenosine signaling indices and SZ symptomatology. We used BPRS-P and SAPS as positive symptom scales, BPRS-N and SANS as negative symptom scales, and the GAS as a measure of global functioning. Testing relationship between cognitive symptoms and adenosine signaling was beyond the scope of this study. Results yield interesting information which had not been reported in previous literatures.

In the FEANS group, significant inverse correlations between adenosine and positive symptoms supported the adenosine theory. It implied that lower adenosine levels correlate with more severe positive symptoms. As expected, ADA was found to correlate positively with positive symptoms, meaning that higher ADA activity levels predict more severe positive symptoms. In addition, inosine levels also inversely correlate with BPRS-P. Since previous studies have established adenosine's role in regulating dopaminergic signaling (S. Ferré et al. 1991a, S. Ferré et al. 1991b, Salmi et al. 2005) via ARs, and positive symptoms are closely related to the dopaminergic alterations (Davis and Kahn. 1991, Howes and Kapur. 2009), we have reason to believe that adenosine signaling deficits may, at least partially contribute to SZ positive symptoms through dopaminergic signaling. Adenosine and ADA activity levels also successfully predict global functioning assessment scores that reflect the extent to which symptoms exist and impair or impede daily functioning. This finding implied that adenosine

signaling deficits are associated with the extent to which symptoms impair patient daily functioning (including work, school, social and personal self-care).

Surprisingly, we didn't find any significant correlations between adenosine signaling and negative symptoms. Negative symptoms has been linked to glutamatergic signaling alterations in SZ (Coyle and Tsai. 2004, Farber. 2003). However, since glutamatergic signaling was not measured in this dissertation, we are not able to check whether it can be affected by adenosine or not. Overall, our findings regard to adenosine signaling and negative symptoms did not support the adenosine theory and suggested that adenosine signaling might not directly contribute to negative symptoms.

In the chronically ill, off-treatment SZ group, there were findings similar to those for the FEANS group. Adenosine inversely correlated with positive symptoms while insignificantly correlated with negative symptoms. The only difference is that significant inverse correlations was observed between inosine and positive symptoms for the FEANS group, but not for the chronic group. This difference might be caused by the limited sample size of the chronic group in comparison with the FEANS group. Also, we did not find difference of correlating patterns between CSF analyte levels-symptomatology and plasma analyte levels-symptomatology, which divergently validated findings in 2.3.1.

7.5 CONCLUSION

1. Adenosine, inosine and ADA activity levels each significantly correlated with positive symptoms in both FEANS and chronic off-treatment SZ group. Also, adenosine and ADA significantly correlated with GAS. These findings support the adenosine theory that adenosine signaling deficits contribute, at least in part, to SZ symptomatology.
2. No significant correlation was observed between adenosine signaling and negative symptoms, suggesting that adenosine signaling deficits in SZ may not directly contribute to negative symptoms.

8.0 SUMMARY AND PERSPECTIVE

Based on all information that were yielded from this dissertation and other literatures, here is the proposed model in explaining part of SZ's pathophysiology (Figure 34): Due to the environmental and genetic factors (Tsuang. 2000, Tsuang et al. 2001), oxidative stress level in the body was elevated (Boskovic et al. 2011). Accumulated oxidative stress levels led to the breakdown of AODS (Othmen et al. 2008, Reddy et al. 2003). As an important component of AODS, uric acid level thus was reduced (Yao and Zhou. 2017, Yao et al. 1998). The reduction of uric acid levels led to the compensatory response of the purine pathway and the metabolism was shifted from adenosine towards uric acid (Yao and Zhou. 2017) and thus the adenosine signaling on the other end of the metabolism was compromised and deficits were formed (Cai et al. 2017, Cai et al. 2018). Then the inhibitory effect from ARs on DRs in the CNS was reduced. Due to the decreased inhibitory effect of adenosine signaling on striatal dopaminergic signaling via ARs-DRs interactions, striatal dopaminergic signaling was increased (Ferré et al. 1994). Increased dopaminergic signaling in the striatal region resulted in positive symptoms of SZ, which have been illustrated by numerous literatures (Davis and Kahn. 1991, Howes and Kapur. 2009). On the other hand, increased oxidative stress led to increased lipid peroxidation on cell membranes (Khan et al. 2002, Herken et al. 2001). As adenosine signaling decreased, the A₁R's up-regulation on insulin activity through cAMP-dependent protein kinase pathway was also diminished (Koupenova et al. 2013). Thus the inhibitory effect of insulin on membrane PLA₂ activity through phosphoinositide 3-kinases pathway was weakened (Thong

et al. 2007, Eichhorn et al. 2001). Consequently, lipolysis rate through membrane PLA₂ was increased (Zhou et al. 2017, Zhou et al. 2018). Increased lipolysis and lipid peroxidation on cell membranes result in membrane defects and eventually caused positive symptoms and impaired global functioning. Current front-line treatments for SZ, including first- and second-generation antipsychotics, were mainly designed to block D₂ receptors, and thus explained that most positive symptoms are responsive to the current front-line antipsychotics while negative and cognitive symptoms are not. In this dissertation, we also noticed that current antipsychotics cannot fully normalize the disrupted adenosine signaling in SZ, which may contribute to the weakness of current antipsychotics in treating refractory symptoms in SZ patients.

Findings in this dissertation supported this model by providing several important evidence. First, we observed increased oxidative stress levels and increased lipid peroxidation in SZ patients in section 4.3.5. Decreased total antioxidant status and uric acid levels in the plasma were observed in section 4.3.1 and 4.3.5, suggesting a possible breakdown of AODS in SZ pathology. Second, in section 2.3.2, 2.3.3 and 2.3.5, we observed significantly reduced levels of adenosine in SZ patients from both plasma and CSF samples. Also, we observed significantly decreased inosine and significantly increased ADA activity levels in the CSF samples in SZ patients. This finding suggested that adenosine signaling deficits might present in SZ patients. Third, section 4.3.1, 4.3.2 and 4.3.3 supported the model by showing a homeostatic imbalance of purine pathway in SZ patients. Fourth, membrane defects and striatal hyperdopaminergia were partially supported and significantly correlated with adenosine levels based on findings in chapter 5 and chapter 6. Fifth, from section 7.3.1 and 7.3.2, we observed

significant associations between positive symptoms and analytes (adenosine, inosine and ADA). We also observed significant associations between global functions and these analytes.

This proposed model may help us to better understand SZ's pathophysiology and point out the clinical significance of adenosine signaling in diagnosing and treating SZ patients. First, based on findings, plasma and CSF adenosine level can serve as important biomarker and therapeutic indicator of SZ. However, plasma ADA and inosine levels cannot serve as biomarkers and therapeutic indicators of SZ due to increased variance and noisiness in the peripheral tissues. For the CSF samples, despite significant correlations between CSF and plasma analyte levels were observed in most cases, levels of ADA and inosine in the CSF were observed having less variance and significantly different with those in controls. Also, it was observed that levels of ADA and inosine in the CSF could be significantly affected by antipsychotics. It is speculated that SZ mainly affects adenosine signaling in the CNS and antipsychotics can normalize adenosine signaling more in the CNS than in peripheral tissues. Therefore, ADA activity and inosine levels in the CSF can still serve as biomarkers and therapeutic indicators of SZ. Second, findings suggested that adenosine signaling modulator might be the silver lining in the development of new generation of antipsychotics.

There are still a lot need to be studied in this area. This dissertation is standing on the theoretical foundation of the adenosine theory (Boison et al. 2012, Lara et al. 2006). Based on our observations, the biggest concern comes from the relationship between adenosine signaling and SZ negative symptoms. Based on adenosine theory, adenosine signaling should at least partially correlate with negative symptoms. However, we didn't observe significant

correlations between adenosine signaling and any negative symptom scale scores in either FEANS or chronic off-treatment SZ group. The adenosine theory claimed that adenosine signaling connected to negative symptoms through glutamatergic signaling with interactions between ARs-NMDARs (Tebano et al. 2005, Sebastião et al. 2000, Nörenberg et al. 1997). Since we did not measure metabolites in glutamate pathway in this dissertation, more studies are needed to illustrate the relationship between these two pathways.

Besides glutamatergic signaling, serotonin theory is also an important component in interpreting SZ's pathology. However, there is few paper explored the relationship between serotonin and adenosine signaling. Actually, serotonin receptors have not been fully studied yet. Basic molecular pharmacology data is needed to support the serotonin theory of SZ.

Another interesting topic to study is the cognitive symptoms in SZ. Since we did not have cognitive symptoms scale data on hand, it would be interesting to link adenosine signaling to cognitive symptoms in SZ.

Overall, adenosine signaling and purine metabolism seem to have long been overlooked in the SZ research. As adenosine theory further develops and more clinical studies that focusing on it will be carrying out during these years, more and more promising findings are expected to come out in the near future.

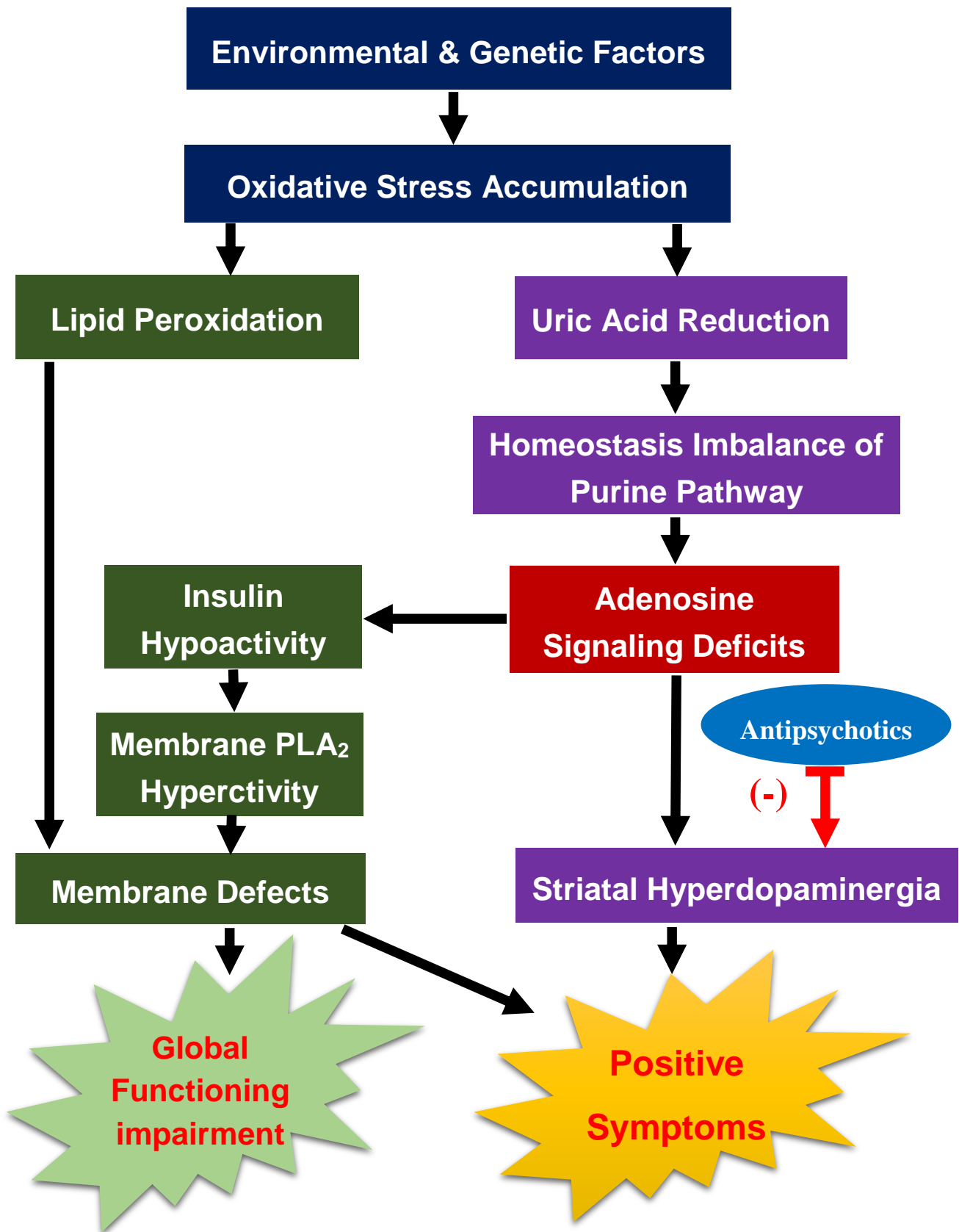


Figure 35. Postulated model of adenosine signaling in the SZ pathophysiology.

Abbreviation: PLA₂ = phospholipase A2.

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